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Research on the Synthesis Process of the Key Intermediate T18 in Nirmatrelvir

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ABSTRACT

The development of antiviral drugs against COVID-19 has attracted significant interest in recent years. Paxlovid, developed by Pfizer, is an orally administered small molecule drug for the treatment of COVID-19. It has attracted widespread attention due to its outstanding clinical performance. Nirmatrelvir, as the active ingredient of Paxlovid, has a huge market demand. Therefore, the development of a mild, efficient, and industrially feasible synthesis method for Nirmatrelvir is meaningful. This study investigates the synthesis process of the key amide intermediate T18 in Nirmatrelvir. Various amide synthesis methods were attempted, and it was determined that the best result can be achieved by using HOPO as a catalyst and using EDCI as condensing agent. Based on this, detailed optimization of reaction parameters was carried out to further reduce the amount of HOPO and eliminate the use of ethyl acetate, methyl tert-butyl ether, brine and magnesium sulfate. Additionally, the post-treatment process was simplified, and a crystallization process more suitable for industrial production requirements was developed. These findings provide important technical support for the industrial production of Nirmatrelvir.

Introduction

Background Information

At the end of 2019, the novel coronavirus epidemic swept the world. As of November 2022, more than 600 million people have been infected with the virus worldwide, with the death toll reaching 6.6 million, accounting for 1.039 % of the number of infections[1]. At the beginning of the epidemic, many novel coronavirus vaccines were developed to prevent infection. However, with the increasing number of confirmed cases, the development of drugs for the treatment of novel coronavirus infections has become urgent. At present, some drugs have been approved for the treatment of novel coronavirus infections, including Paxlovid [2] of Pfizer, Molnupiravir [3] of Merck and Azvudine [4] of real organisms.

Among them, Paxlovid developed by Pfizer is an oral small molecule novel coronavirus treatment drug, which can be used to treat patients with mild to moderate novel coronavirus pneumonia, especially for patients with severe high-risk factors. According to the results of Pfizer 's clinical trials, Paxlovid has outstanding clinical advantages in the treatment of non-hospitalized and high-risk patients with COVID-19. For patients who developed symptoms within three days and received timely treatment, Paxlovid reduced the risk of death by 89 % compared with placebo. As of 2022, Paxlovid has obtained more than 40 listing licenses worldwide, and its sales have reached USD 18.933 billion. This shows that Paxlovid has been widely used around the world and has received a good market response.

Paxlovid is a compound preparation of nematavir and ritonavir, which is mainly composed of nematavir and ritonavir. Among them, Naimatwe, (1R, 2S, 5S) -N- ((S) -1-cyano-2- ((S) -2-oxopyrrolidin-3-yl) ethyl) -3- ((S) -3,3-dimethyl-2- (2,2,2-trifluoroacetylamino) butyryl) -6,6-dimethyl-3-azabicyclo [3.1.0] hexane-2-carboxamide, is the main component, as Figure 1-1 shows, it can inhibit the 3CLpro enzyme of the

novel coronavirus to block the replication of the virus and reduce the viral load in the body. Ritonavir is a cytochrome P450 inhibitor. Nematvir is rapidly metabolized by cytochrome P450 in the liver after entering the human body. Ritonavir can prolong the residence time of nematvir in the human body, increase the concentration of nematvir in the human body, and improve the therapeutic effect.

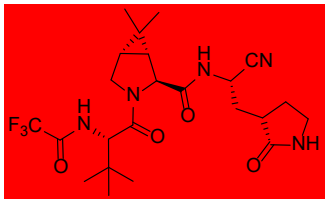


Figure 1. Structure of nematavir

The Synthesis Root of Nirmatrelvir

Nematavir molecule contains six chiral centers and four amide groups. In 2022, Pfizer announced a synthetic route for the production of nematavir[2], as shown in Figure 1-2 .

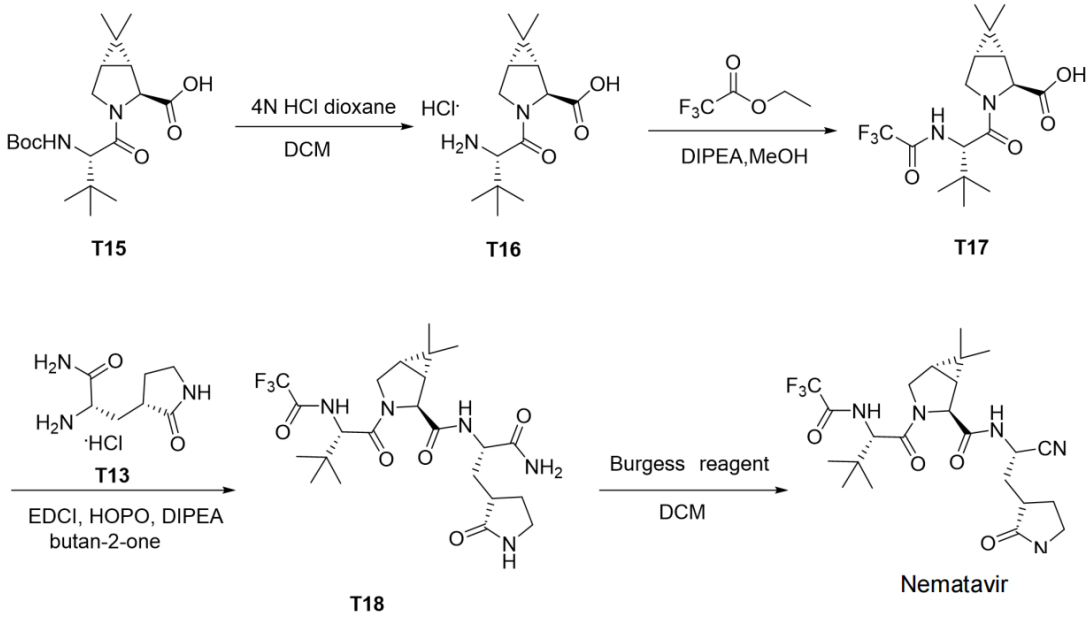


Figure 2. synthesis process of nematavir

Pfizer 's reported synthetic route of nematavir is starting from T15.T15 first removes Boc protection under hydrochloric acid conditions to obtain intermediate T16.T16 then reacts with ethyl trifluoroacetate to form T17.Then T17 and T13 are subjected to condensation reaction to obtain T18.Finally, the amide group in T18 is converted into a cyano group using Burgess reagent dehydration to obtain nematavir. In this process, the key intermediate T18 is formed by the condensation of two key chiral intermediates T17 and T13 under the action of EDCI, using 2-butanone as the reaction solvent and DIPEA as the base. The use of condensation reagents may affect the conversion rate and chiral purity of T18, which in turn affects the cost and quality of nematavir.

The condensation reaction of T17 and T13 is the synthesis reaction of amide. The amide bond is one of the important functional groups commonly found in biomolecules and drugs, accounting for about 25 % of the total number of reaction types in listed drugs. The [5] amide bond can not only be used as a pharmacophore, but also as a connecting group. Due to the wide application of amide bonds, its synthesis method has also received extensive attention. The most ideal amide synthesis method is to directly form amides by condensation of carboxylic acids and amines, and only water is produced as a by-product. However, due to the limited reactivity, the amidation reaction is usually difficult and often requires heating or water separation to promote the reaction. In addition, activation reagents can also be used to improve the activity of carboxyl groups. The commonly used activation methods mainly include acyl chloride method, sulfonyl ester method, mixed anhydride method and condensation agent condensation method. [6-8] Using different methods, the reaction effect and post-processing methods will be different. Considering the huge market potential of nematavir, it is of great research significance and application value to develop an efficient, mild and industrially feasible method to synthesize T18.

The Study of the Synthesis Process

According to the method reported by Pfizer for the preparation of nemathvir, we prepared and purchased the corresponding materials and reagents, and repeated the literature methods. The results are listed in Table 2-1 :

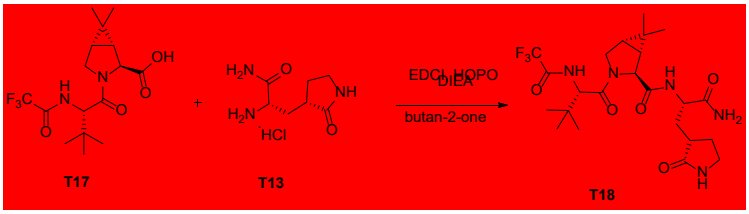


Figure 3. T18's synthesis reaction

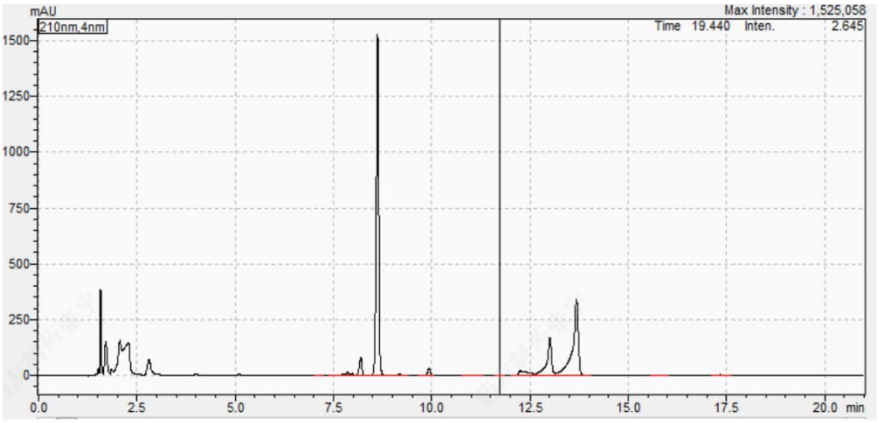
Table 1. repeat results [a]

Number	HOPO(e.q)	T17 left (%)[b]	T18 purity(%)[b]	yield (%)[c]	T18 purity (%)[c]
1	0.3	0.18	51.21	--	--
2	0.7	1.4	84.01	56.19	84.85

Reaction condition : 1.0 eq T17, 1.2 eq T13, 1.2eq EDCI, 3.0 eq DIEA, 11 V/m 2-butanone, react overnight at room temperature ; [b] liquid detection result ; [c] post-treatment result .

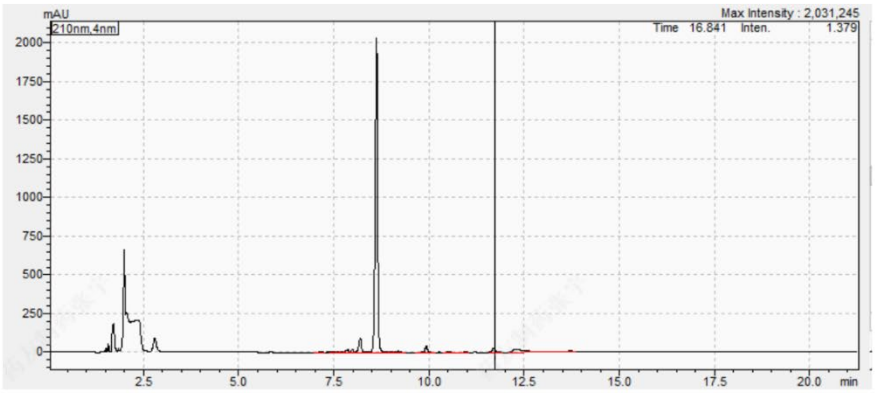
After our attempt, we found that the method reported by Pfizer has some difficulties in reproducibility. The main problem are as follows: 1) The reaction is complex, and the purity of the product is only 51.21 % (Number 1); 2) when using 2-butanone as a solvent, post-processing solvent emulsify during washing process. And because of its good water solubility, there are still a large number of products remaining in the water layer

after extraction, resulting in a large amount of product loss. After complex post-processing steps, we only obtained the target product with a yield of 56.19 % and a purity of 84.85 % (Number 2). In order to improve the above problems, we first tried to increase the amount of HOPO. When the amount of HOPO was adjusted from 0.3 to 0.7 equivalent, the raw material could be basically completely converted, and the purity of the product reached 84.01 % (Number 2). This gave me great encouragement and added confidence to the follow-up program.



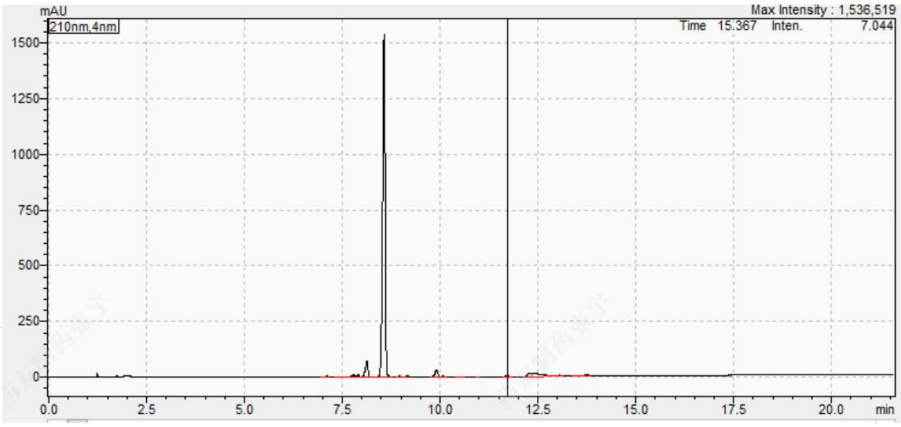
Peak#	Ret. Time	Area%
1	7.172	0.10
2	7.520	0.15
3	7.618	0.11
4	7.737	0.19
5	7.869	0.67
6	7.983	0.34
7	8.196	3.27
8	8.623	51.21
9	8.998	0.05
10	9.188	0.35
11	9.931	1.34
12	10.966	0.05
13	11.257	0.09
14	11.702	0.18
15	12.261	2.97
16	13.008	11.46
17	13.683	27.25
18	15.731	0.08
19	17.338	0.15
Total		100.00

Graph 1. Patent method (0.3e.q. HOPO)



Peak#	Ret. Time	Area%
1	7.167	0.17
2	7.517	0.21
3	7.616	0.12
4	7.737	0.25
5	7.868	0.97
6	7.982	0.65
7	8.194	4.18
8	8.621	84.01
9	9.001	0.14
10	9.186	0.38
11	9.929	1.68
12	10.548	0.06
13	10.964	0.06
14	11.703	1.40
15	12.261	4.17
16	13.020	0.53
17	13.715	1.04
Total		100.00

Graph 2. Patent method (0.7e.q. HOPO)



Peak#	Ret. Time	Area%
1	7.106	0.29
2	7.447	0.08
3	7.548	0.07
4	7.666	0.18
5	7.796	0.77
6	7.911	0.61
7	8.127	4.14
8	8.245	0.07
9	8.568	84.85
10	8.956	0.18
11	9.157	0.38
12	9.907	1.96
13	10.074	0.16
14	10.541	0.05
15	10.978	0.06
16	11.714	0.19
17	12.290	1.42
18	12.434	3.31
19	13.050	0.43
20	13.745	0.81
Total		100.00

Graph 3. Product after post-treatment (patent method with 0.7 e.q. HOPO)

In view of the diversity of amide synthesis methods, different methods may lead to different conversion rates and reaction solution purity. Moreover, the reagents used are different, and the operation mode may also be simplified. Inspired by this, we screened the common amide synthesis methods, hoping to find a method with good conversion rate and high purity of the reaction solution to more effectively prepare the key intermediate T18. After determining the appropriate method, we also hope to optimize the post-processing method to simplify the preparation process as much as possible and make it more in line with the needs of industrial production.

Comparing Different Intermediate T18's Synthesis Methods

Direct Condensation

We first used toluene as the reaction solvent and tried two reaction substrates T17 and T13 to react directly. Taking advantage of the characteristics that toluene and water can form azeotrope with low boiling point, the azeotrope is refluxed and evaporated by heating. Due to the incompatibility of toluene and water, the evaporated water and toluene can be stratified, and the generated water can be removed by a condensation water separation device to retain toluene. Due to the reduction of water, the reaction equilibrium will move in the direction of T18 formation. However, in the experimental attempts, we found that the reactivity of T17 and T13 was low, and both T17 and T13 were insoluble in toluene, which made the reaction difficult to occur. Under heating conditions, we did not observe obvious water formation and could not form an azeotrope with toluene. Therefore, we excluded this preparation method.

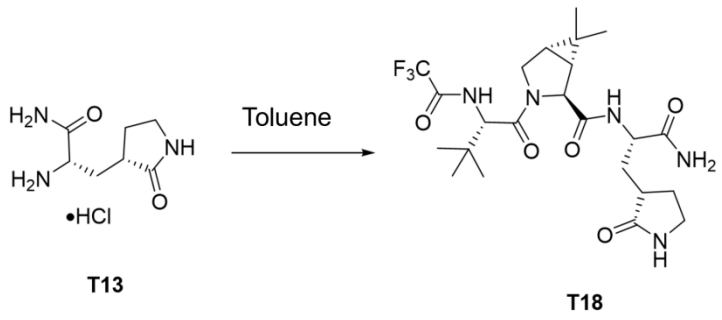


Figure 4. Direct condensation method

Acyl Chloride Method

The synthesis of amides by acyl chloride method is a common synthesis method, which can be used to synthesize a variety of amide compounds and has a wide range of applications in the fields of drug synthesis, organic synthesis and material science. This method is mainly reacted through the carboxylic acid and acylation reagents such as : thionyl chloride, oxalyl chloride, phosphorus trichloride, etc., to generate acyl chloride, and then react with amines to form amide compounds, while generating hydrogen chloride as a by-product. The advantage of acyl chloride method for the synthesis of amides is that the reaction rate is fast, and the reaction usually takes only a few hours to complete. Since acyl chloride can react with a variety of amine substrates, this method can also be used to synthesize amides with high activity.

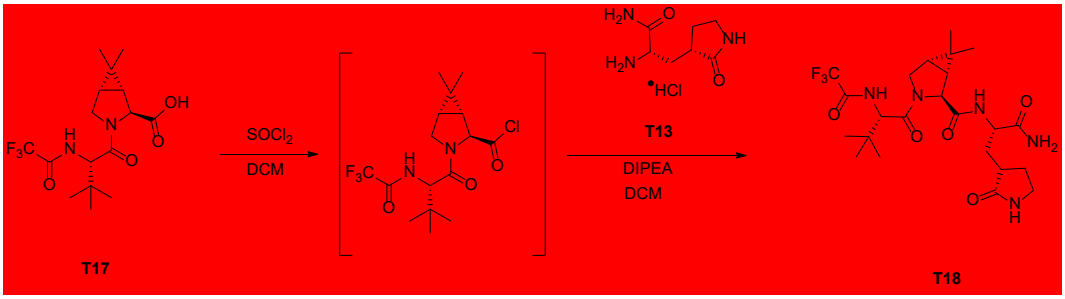


Figure 5. Acyl chloride method

With reference to the patent CN 114213275A [9], we chose thionyl chloride as the acylation reagent for the reaction. However, after testing, the method has 90.13 % of the T17 remaining, the purity of the product T18 is only 9.08 %, and the reaction conversion rate is very poor. Considering that acyl chloride is highly corrosive and toxic, strict protective measures need to be taken in the experiment. Based on these considerations, we decided not to continue to use this method for subsequent experiments.

Mixed Anhydride Method

The theory of mixed anhydride method is to first form anhydride from acid, then react with amine to form amide compounds, and generate acid as a by-product at the same time. The mixed anhydride method is suitable for the synthesis of different types of amides, including fatty amides and aromatic amides, because of its mild reaction conditions and no need for strong acid or alkali.

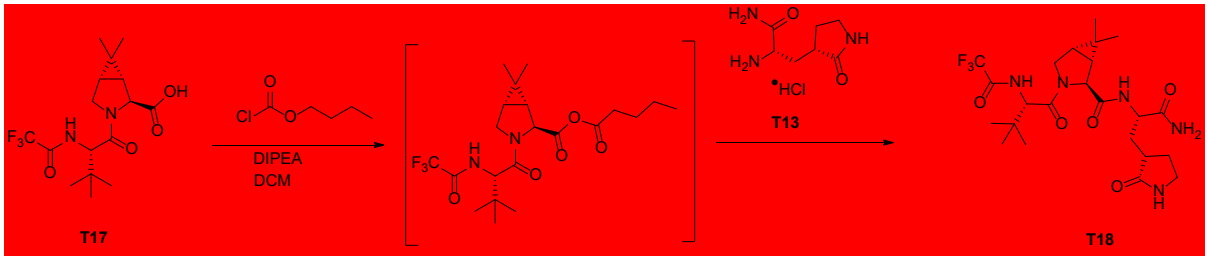


Figure 6. mixed anhydride method

We chose butyl chloroformate as the reaction reagent to try the reaction. After trying, we found that the conversion rate of the raw material was not ideal, and the raw material still remained 29.20 % after the reaction. Secondly, the active ester reacted with T13 may also have some side reactions, resulting in the main peak purity of only 60.45 %. Based on these results, we believe that the advantages of this method are not obvious enough, so we no longer consider using this method to prepare T18.

Sulfonyl Easter Method

Sulfonyl chloride (such as methanesulfonyl chloride, benzenesulfonyl chloride, p-toluenesulfonyl chloride, etc.) are easy to form sulfonyl esters with acid under alkaline conditions. Sulfonyl ester is a group that is easy to leave. It is easy to react with nucleophiles to undergo condensation reaction and drop a molecule of acid. Based on this property, sulfonyl chloride is also widely used in the synthesis of amide bonds.

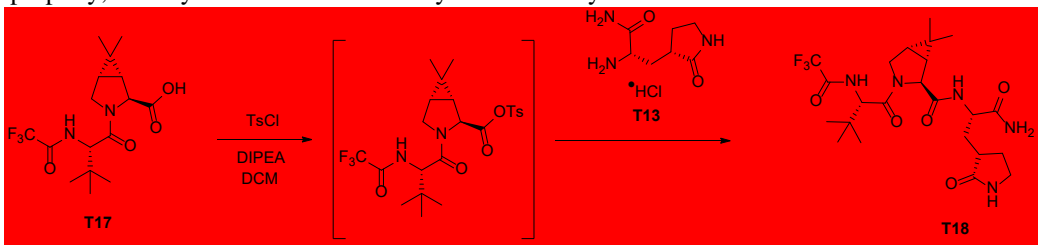


Figure 7. Sulfonyl easter method

According to this principle, we chose p-toluenesulfonyl chloride as the reaction reagent, dichloromethane as the solvent, and diisopropylethylamine as the base to activate the T17 carboxyl group to form a sulfonyl ester. Through the test results of the reaction solution, it can be seen that the conversion rate of the reaction is still poor, and the raw material has 18.11 % left after the reaction. In addition, due to the presence of multiple amino competition sites in T13, it is easy to produce by-products, there are still 26.01 % impurities in the case of a large amount of raw materials remaining. This will undoubtedly increase the cost of production and the difficulty of purification. Therefore, we decided not to use this method for further attempts.

Using Condensing Agent

Condensation agent is a kind of compound commonly used in the synthesis of amide compounds. In the reaction, they can react with acid to form an active intermediate, which is then condensed with the amine substrate to form an amide compound. Such reactions are usually carried out in inert solvents. The advantage of the condensation reagent method is that the reaction conditions are relatively mild and the applicability is wide, so it is widely used in the field of amide synthesis. Among them, onium salts and carbodiimides condensing agent are the most commonly used.

Onium Salt Condensing Agent

Common onium salt condensation reagents include HATU (O- (7-azabenzotriazole-1-yl) -bis (dimethylamino) carbonium hexafluorophosphate), HBTU (O- (benzotriazole-1-yl) -bis (dimethylamino) carbonium hexafluorophosphate) and TBTU (O- (benzotriazole-1-yl) -bis (dimethylamino) carbonium tetrafluoroborate). The theory of the onium salt condensing agents is that in the reaction, they react with the acid to form an active intermediate, usually an onium hydrochloride or an onium hydrochloride ester, and then react with the amine substrate to form an amide. Due to the high nucleophilicity and activity of the onium salt condensing agent, it can promote the formation of amides, making such reactions often more efficient.

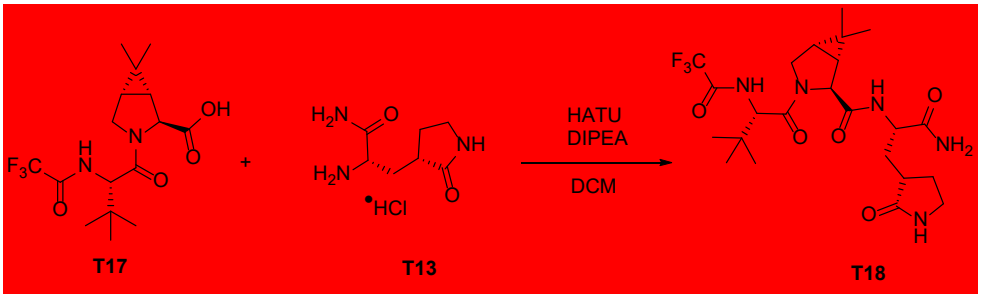


Figure 8. Sing HATU as condensing agent

Due to its strong activity, we chose the HATU as the condensation agent to try the reaction. The results showed that there are 8.89 % of the raw material remaining and the purity of the main peak was 76.92 %, 15 % of the impurities were generated. Since there were many kinds of impurities and miscellaneous components. Compared with the method using EDCI as the condensation agent (the percentage of remaining raw material is 1.4 %, the purity of the main peak is 84.01 %), the conversion rate of the raw material and the purity of the main peak are poor. In addition, HATU is more expensive than EDCI. The selection of HATU as a condensation reagent will further increase the material cost of T18. Therefore, we no longer consider further optimization of the method.

Carbodiimide Condensing Agent

Except the EDCI (1- (3-dimethylaminopropyl) -3-ethylcarbodiimide) reported by Pfizer, the most widely used carbodiimide condensation agents are DIC (diisopropylcarbodiimide) and DCC (dicyclohexylcarbodiimide). When such condensation agents are used for reactions, activators such as HOPO (2-hydroxypyridine-N-oxide), HOBt (1-hydroxybenzotriazole) or DMAP (4-N, N-dimethylpyridine) are usually required. This is mainly because the stability of the intermediate is poor after the addition of acid and carbodiimide to form an active intermediate. If it is further converted into the corresponding active ester or active amide without using an activator, the intermediate will be rearranged to form a urea by-product.

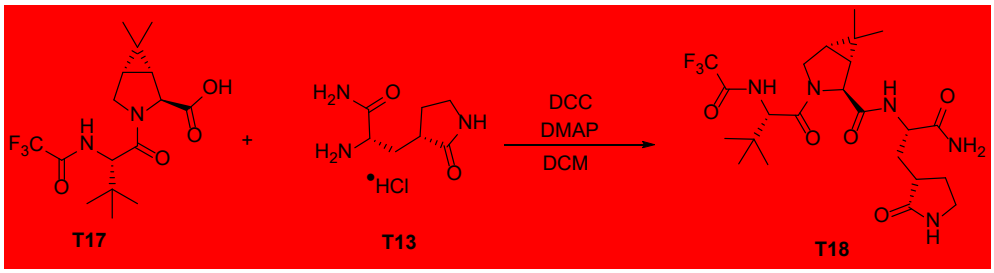


Figure 9. using DCC as condensing agent

Since the price of DCC is relatively cheap, we also used it as a condensation agent for reaction screening. Considering that DCC needs to be used in conjunction with DMAP, we tried to use DCC plus DMAP instead of the combination of EDCI and HOPO reported by Pfizer. However, according to the analysis results, the purity and conversion efficiency of the obtained reaction solution were low, the purity of the main peak was only 27.23 %, and the remaining amount of raw materials was 47.7 %. In addition, compared with EDCI, the use of DCC has an obvious disadvantage, that is, the by-product of the condensation reaction, dicyclohexylurea, is difficult to be completely removed by conventional purification methods. Based on the above considerations, we decided not to continue to optimize the system.

Nodule

We tried several common amide synthesis methods to synthesize the key intermediate T18 of nematvir. According to the experimental results, the substrate activity of T13 and T17 is low, and the direct condensation method cannot be used for the reaction. Although acyl chloride method, mixed anhydride method and sulfonyl ester method can be used to produce products, most methods have problems such as low conversion rate of raw materials and poor chemical selectivity, resulting in low product purity and high impurity content. Moderate results can be obtained by using the condensation agent method, but the cost of HATU condensation agent is high, and DCC as a condensation agent will produce problems such as by-products that are difficult to remove.

At the same time, by optimizing the method reported by Pfizer, we increased the amount of activator HOPO to 0.7 equivalent, and obtained a higher conversion rate and a reaction purity of 84.01 %. Therefore, compared with the above method, this optimized process has advantages in conversion rate and reaction purity. Based on these considerations, we decided to continue to use EDCI as a condensation reagent, HOPO as an activator, 2-butanone as a solvent, and diisopropylethylamine as a base as the optimal reaction system, and on this basis, follow-up detailed experimental optimization.

Reaction Optimization

Reaction Solvent Investigation

After determining the reaction system, we continued to optimize the parameters of the reaction. Consider that 2-butanone used in the previous experiment had good water solubility, making it necessary to add an additional organic solvent with poor water solubility for extraction during post-treatment, and saturated salt water was added during extraction to reduce the water solubility of 2-butanone and reduce product loss. Based on this problem, we screened other probable reaction solvents and listed the results in Table 2:

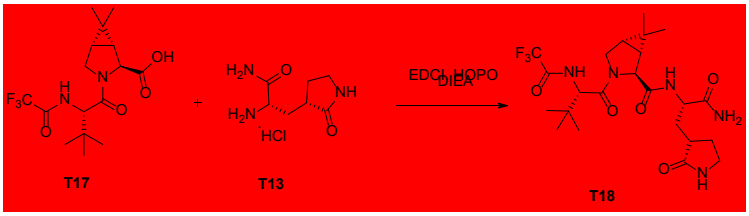


Figure 10. Reaction solvent comparison[a]

Table 2. Reaction solvent comparison[a]

No.	Solvent	T17 remained (%)[b]	T18 purity (%)[b]
1	2-butanone	1.40	84.01
2	dichloromethane	2.40	82.59

React condition：1.0 eq **T17**，1.2 eq **T13**，1.2eq EDCI，0.7eq HOPO，3.0 eq DIEA，11 V/m solvent，react overnight at room temperature；[b]liquid detection result.

It can be seen from the table that when dichloromethane is used as the reaction solvent, the remaining raw materials and the purity of the product are close to those when 2-butanone is used as the solvent. Since the solubility of dichloromethane is poor in water, it may be possible to simplify the post-treatment of the reaction.

Therefore, we decided to choose dichloromethane as the optimal solvent and continue to optimize the subsequent parameters.

HOPO Consumption Investigation

HOPO plays a role in improving the yield and reducing side reactions in the reaction. It does not consume itself, but only participates in the reaction cycle as a catalyst. The amount of HOPO may be reduced after the solvent system is changed. Therefore, we investigated the reaction using different amount of HOPO , and the results are as Table 3:

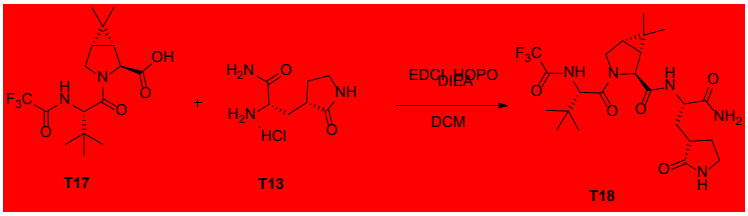


Figure 11. HOPO dosage[a]

Table 3. HOPO dosage[a]

No.	HOPO e.q.	T17 (%) [b]	T18purity (%) [b]
1	0.3	0.05	85.01
2	0.7	2.40	82.59

React condition： 1.0 eq **T17**， 1.2 eq **T13**， 1.2eq EDCI， 3.0 eq DIEA， 11V/m DCM， react overnight in room temperature ； [b] liquid detection result.

It is obvious that when dichloromethane was used as the solvent, the purity of the experimental product using 0.7 equivalent of HOPO was about 82.59 %, and the remaining raw material was 2.40 % (No.2). When using 0.3 equivalent of HOPO, the purity of the experimental product was about 85.01 %, and the remaining raw material was 0.05 % (No.1). This indicates that reducing the amount of HOPO from 0.7 equivalent to 0.3 equivalent has little effect on the reaction. Therefore, we decided to reduce the amount of HOPO to 0.3 equivalent to reduce both cost and agent.

Alkali Quantity Investigation

The addition of alkali to the condensation reaction is to help promote the reaction, in this case, alkali is also necessary to neutralize a molecule of hydrochloric acid contained in T13. In order to determine the optimal amount of alkali, we investigated the amount of alkali, and set the equivalent of diisopropylethylamine to 1.5,2.0 and 3.0 equivalents for comparative experiments. The results are as:

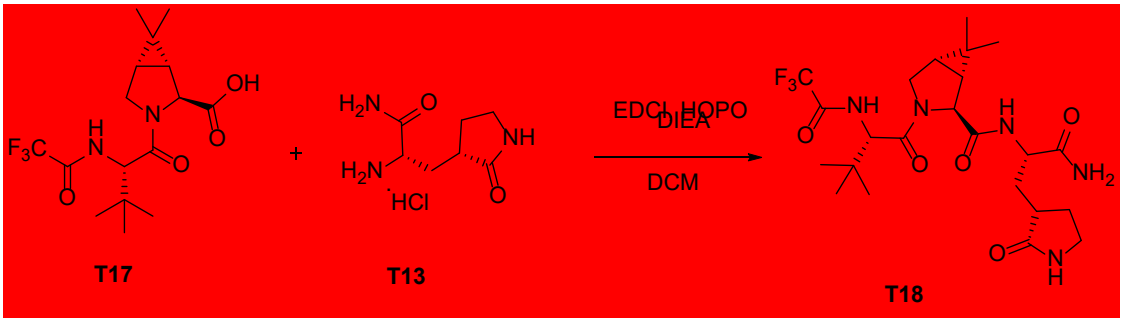


Figure 12. alkali quantity[a]

Table 4. alkali quantity [a]

No.	Alkali (e.q)	T17 remains (%)[b]	T18 remains (%)[b]
1	1.5	0.10	82.87
2	2.0	0.23	81.69
3	3.0	0.05	85.01

React condition：1.0 eq **T17**，1.2 eq **T13**，1.2 eq EDCI，0.3 eq HOPO，11V/m DCM，react overnight at room temperature；[b] liquid detection result

Through liquid phase detection, we obtained the following results : the purity of the reaction solution No.1 was 82.87 %, and the remaining raw material was 0.10 %; the purity of the reaction solution No.2 was 81.96 %, and the remaining raw material was 0.23%. Compared with the results of adding 3.0 equivalents of diisopropylethylamine, although the raw materials can be completely converted, 1.5 and 2.0 equivalents generate more impurities and the product purity is lower. Therefore, we decided not to change the amount of bases.

Optimization of Post-Processing Method

The post-treatment method reported by Pfizer involves the addition of methyl tert-butyl ether and ethyl acetate for extraction and washing, and brines is also needed to reduce the water solubility of 2-butanone so as to reduce the loss of the product during the process. Due to the poor water solubility of dichloromethane, during the extraction process, we removed the use of ethyl acetate and methyl tert-butyl ether, and replaced the salt water with water for extraction. This improvement turned out to be successful.

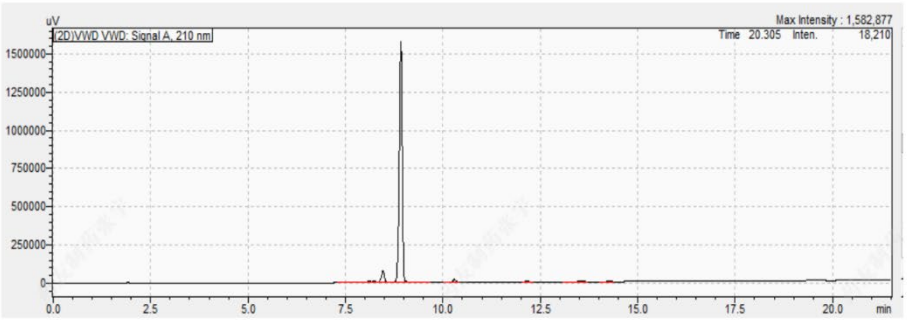
After the extraction is completed, Pfizer reported that the product is obtained through drying, suction filtration and rotary evaporation steps. However, these steps do not apply to mass production due to its cost. Therefore, we hope to improve these steps to an anti-solvent crystallization method that is more suitable for industrial production. Based on this, we investigated the crystallization solvent, and the results are listed in Table 5:

Table 5. Crystallization solvent

No.	Crystallization solvent	Precipitation
1 [a]	methyl tertiary-butyl ether	No
2 [a]	normal heptane	yes（emulsify）
3[b]	normal heptane	yes

Crystallization condition： [a] drip the antisolvent into organic phase； [b] drip the organic phase into the anti-solvent 。

After testing, we found that there was no solid precipitation when methyl tert-butyl ether was added to the organic phase after extraction (No. 1). Although the organic phase of n-heptane has solid precipitation, the curing effect is not ideal under this method. It is easy to emulsify during the crystallization process of dripping n-heptane. Even if the dripping velocity is controlled, the emulsification phenomenon cannot be avoided. Based on this, we further optimized the dripping order, and changed the crystallization method from dripping n-heptane into the organic phase to the method of dripping the organic phase into n-heptane, thus successfully solving the problem of crystallization emulsification. After such optimization, the white powder product can be obtained by simple suction filtration, which makes the post-treatment method more in line with the needs of industrial production.



Peak#	Ret. Time	Area%
1	7.769	0.13
2	7.855	0.06
3	7.981	0.19
4	8.123	0.85
5	8.244	0.63
6	8.465	4.76
7	8.592	0.10
8	8.922	90.75
9	9.188	0.06
10	9.332	0.15
11	9.523	0.19
12	10.297	1.11
13	12.156	0.35
14	13.294	0.09
15	13.576	0.29
16	14.290	0.30
Total		100.00

Graph 4. product after post-treatment (improved method)

The Synthesis of T18

After experiments, we determined the best optimal reaction : 1 equivalent of substrate T17, 1.2 equivalent of substrate T13, 1.2 equivalent of EDCI, 0.3 equivalent of HOPO, 11 times the volume of DCM, reacted under room temperature. Under these new conditions, we repeated the reaction and carried out post-processing according to our optimized post-processing method. Finally, after the reaction was completed, we simply washed the reaction solution with water, acid and saturated salt water. Then, the washed organic phase was dripped into n-heptane, and the target product was obtained in a yield of 90.00 % and a purity of 90.75 %, far exceeding the process reported by Pfizer.

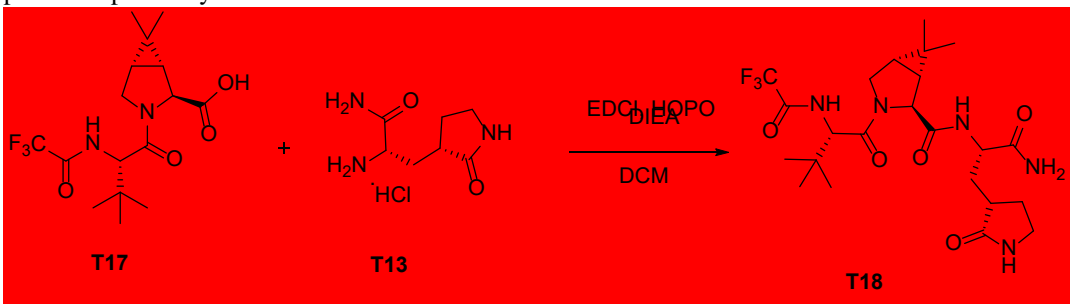


Figure 13. T18 synthesis

Nodule

After improving the reaction solvent, the amount of HOPO, alkali and the post-treatment method, we successfully replaced the reaction solvent from 2-butanone to dichloromethane. After substitution, we found that the amount of HOPO can be reduced from 0.7 equivalent to 0.3 equivalent with out affecting the reaction result. This improvement significantly reduces the material cost in the production process.

In terms of post-processing methods, we eliminated the use of methyl tert-butyl ether and ethyl acetate, and successfully replaced the use of brine with water. This improvement not only reduces the production cost, but also simplifies the complex post-treatment extraction process. At the same time, we explored a method to directly solidify and crystallize the product to replace the traditional process steps with high energy consumption and poor industrial feasibility which include drying, suction filtration and rotary evaporation. Through this direct crystallization method, we can solidify the product from the reaction solution without magnesium sulfate for drying and energy-consuming concentration steps. This not only saves energy, but also simplifies the post-processing steps, making the process more practical and feasible.

Finally, we carried out the experimental verification of the optimized optimal conditions, and recieve the results consistent with the screening process. This result provides an important experimental basis and technical support for the industrial production of the process. Through these optimization measures, we can better achieve the synthesis of the key intermediate T18 of nematvir with high yield and high purity, also improving production efficiency and reducing production costs.

Experiment Part

Apparatus and Reagent

Apparatus used: Rotary evaporator, vacuum drying oven, electronic balance, magnetic stirrer are all provide by Chongqing Yaoyou Pharmaceutical Co., Ltd.

Reagent Name	Molecular Mass	Manufacturer
2-butanone	72.11	Chongqing Chuandong Chemical Co., Ltd
toluene	92.14	Chongqing Chuandong Chemical Co., Ltd
sulfur oxychloride	118.97	ChengDu Chron Chemicals Co,.Ltd
dichloromethane	84.93	Chongqing Chuandong Chemical Co., Ltd
Normal heptane	100.20	Chongqing Chuandong Chemical Co., Ltd
methyl tertiary-butyl ether	88.15	Chongqing Chuandong Chemical Co., Ltd
tosyl chloride	190.65	Anneji (Shanghai) Pharmaceutical Chemical Co., Ltd
N,N-diisopropylethylamine	129.24	Anneji (Shanghai) Pharmaceutical Chemical Co., Ltd
butyl chlorocarbonate	136.58	adamas
HOPO	111.10	Bide Pharmatech Co., Ltd
HATU	380.23	Wuxi Asia Peptide Biological Technology Co., Ltd

Table 6. Reagent Reused

EDCI	191.70	Shanghai Macklin Biochemical Co., Ltd
DCC	206.18	Anneji (Shanghai) Pharmaceutical Chemical Co., Ltd
DMAP	122.17	Anneji (Shanghai) Pharmaceutical Chemical Co., Ltd
HCl	36.50	Chongqing Chuandong Chemical Co., Ltd
T13	207.66	Chongqing Remote Chinese Herbal Medicine Co., Ltd.
T17	364.37	Chongqing Yaoyou Pharmaceutical Co., Ltd.

The Synthesis Process of T18

6.68 g T13 and 9.78 g T17 were added to the 500 mL reaction bottle, 108 mL dichloromethane was added and stirred to dissolve. 0.90 g HOPO was then added to the reaction bottle and dissolved. Cool the reaction to 0-5 °C, and add 10.40 g DIPEA into the reaction flask. After stirring for 10 min, 6.18 g EDCI was slowly added. After the addition, the reaction system was heated to room temperature and stirred overnight. After the reaction was completed, 108 mL of purified water was added to the reaction solution for extraction and washing, and we reserve the organic layer. Add 54 mL 1N hydrochloric acid solution to the organic layer, wash, wait for two layer to seperate and retain the organic layer. Add 108 mL saturated salt water to the organic layer, stand and separate the liquid, still retain the organic layer. Organic layer was slowly dripped into a reaction flask containing 216 mL n-heptane, and the solid was precipitating during the dripping process. After the dripping was completed, the reaction solution was stirred for 1 h, then filter the products, the filter cake was collected and dried to obtain a white powder solid.

Conclusion

We have studied the synthesis process of the key intermediate T18 of nematavir, and through the attempt of a variety of commonly used amide synthesis methods, excluding most of the method. Finally determined that under the catalysis of HOPO, the condensation with EDCI can obtain the best results. On this basis, we have carried out subsequent parameter optimization, and made the following innovative progress and breakthrough points:

1. The reaction solvent was replaced by dichloromethane from 2-butanone, which effectively reduced the use of materials in the production process and reduced the amount of HOPO from 0.7 equivalent to 0.3 equivalent.

2. In the process of extraction and washing, the use of organic reagents such as methyl tert-butyl ether and ethyl acetate was successfully eliminated, which reduced the use of organic reagents, making the production process more green and environmentally friendly.

3. The use of water instead of brine in the extraction process makes the extraction process more simplified.

4. A method for solidifying and crystallizing the product was developed, which replaced the traditional drying, suction filtration and rotary evaporation process steps, reducing the production of magnesium sulfate solid waste and reducing energy consumption.

Finally, based on the T18 synthesis process reported by Pfizer, we increased the purity of the reaction solution from 51.21 % to 84.01 % by increasing the amount of activator HOPO. On this basis, after further reaction optimization, the yield of T18 synthesis was successfully increased by 33.81 % (56.19 to 90.00 %), and the purity of the product was increased by 5.9 % (84.85 to 90.75 %). These important advances will provide important technical support and guidance for the subsequent industrial production of nematvir.

Acknowledgments

I am a high school student who was interested in chemistry, it's been a pleasure to have such a chance to establish and study the project of Nematevir. Thus, with the help of my instructor, I had the chance to create several contrasting experiments and was able to get accurate result through liquid detection. Fortunately, the study was successful and I manage to improve and simplify the process. This gladsome result cannot be accomplished without the help of Dr. Zhang and Dr. Zhou. They have helped me gratuitously and help me revise lots of immature ideas. Also, I truly appreciate the chance and the reagents the company had prepared for me.

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