

Phosphine imide reaction in supercritical CO₂: Synthesis of a compound of pharmaceutical interest

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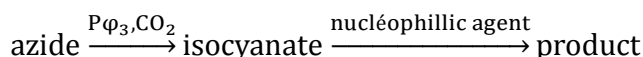
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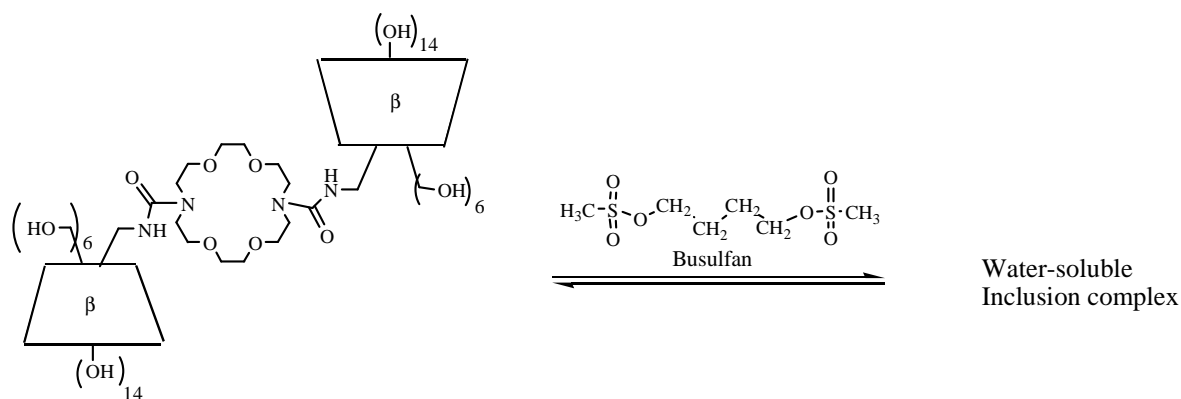
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ABSTRACT

The "phosphine imide" strategy [1] was initially developed in organic solvents to achieve a rapid and easy access to sophisticated cyclodextrines derivatives (urea, carbodiimides and isocyanates) [2]. In this strategy, CO₂ was used as reactant and solvent.



We have previously investigated the usability of supercritical CO₂ (scCO₂) as solvent and reactant for a standard phosphine imide reaction [3] and showed that the production of isocyanates in scCO₂ was following a first order kinetic. In this work, scCO₂ and phosphine imide strategy were used to produce a compound of pharmaceutical interest, which was previously produced in DMF [4]:



The kinetic of this reaction was followed and the desired compound was obtained in less than 2 hours with yield over 92%.

Introduction

Among the various approaches that have been used to improve the solubility and dissolution rate of drugs, complexation with cyclodextrins is one of the most promising ones and this action is even recognized in the Biopharmaceutics Classification System (BCS) [5].

Cyclodextrins are cyclic oligosaccharides that presents like a cone shape with a non-polar cavity and a polar surface. It's this particular configuration that allows the complexation of non-polar guest and in a same time a good solubility of the host-guest complex in water and in biological medium. The cyclodextrins used in most of the studies and applications are natives ones [6] or classical derivatives like DM- β -CyD and HP- β -CyD [7]. But for some compounds, such a simple approach doesn't permit an access to interesting host-guest complexes.

In that case, the use of cyclodextrins doesn't seem an efficient strategy. Using cyclodextrins as parts of more complex structures permits to tune their complexing properties and adapts them to the guest, seems a more successful approach.

Such supramoleculars structures have previously been synthesized in organic solvent using the tandem Staudinger-Aza-Wittg reaction also called "phosphine imide" reaction [8]. The phosphine imide reaction was further developed in $scCO_2$ [9] and the first step was the investigation of a standard reaction that permits obtention of a cyclodextrinyl isocyanate derivative which was a synthon used for a wide range of syntheses [2] [9] [10].

We have investigated the possibility to obtain highly refined supramoleculars compounds by phosphine imide reaction in $scCO_2$. We have chosen a crown ether (**1**) functionalized by two cyclodextrins. After deacetylation compound (**3**) previously showed interesting complexation properties with Busulphan, a widely used anti-leukemia agent [4].

Reactor

A schematic flow-sheet of the equipment is given in Figure 1. Since it was previously described [3], only a short description is reported here. The compression module consists in a CO_2 cylinder, a chiller 1 (3 °C), a piston pump 2 (LEWA EK-M-210V1), a heat exchanger 3 and a Coriolis mass flow-meter (Rheonik REH 07, 0.01 g accuracy). The reaction module consists in a 100 mL vessel 4 (Top Industrie) of internal diameter and height of 40 and 80 mm, respectively. Its temperature is regulated at 0.1 °C by a water circulation.

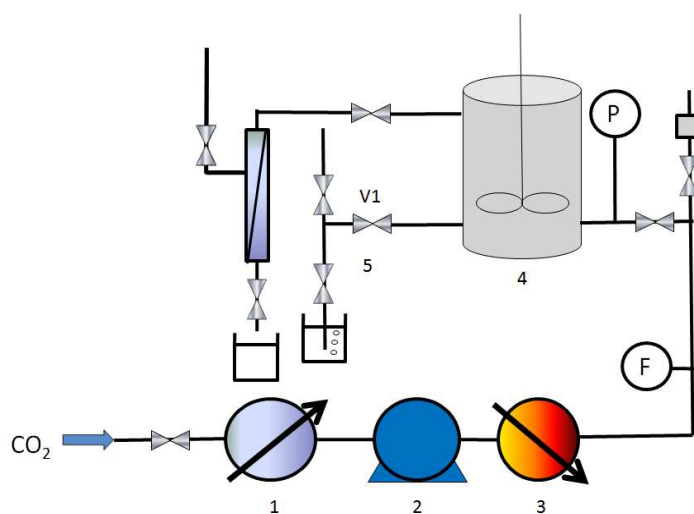


Figure 1: 100 ml high pressure reactor flow sheet

The stirring is provided by a magnetically coupled stirrer with a Rushton-type impeller of 16 mm in diameter located at 5 mm from the vessel bottom; for these experiments, the stirring rate was settled at 1400 rpm. The pressure is measured by a sensor accurate at 0.1 MPa (Touzard et Matignon). The sampling module 5 consists in three valves (Top Industrie) and a pre-calibrated sampling loop of 1 mL; all lines are thermostated by heating strips. Sampling tests conducted using triphenylphosphine at 200bars, 33°C with the stirring device working and concentration between 0.25 and 2.0 g per/liters show good agreements between composition of the sample and composition attempted with a maximal differences of 10% for the lowest concentration [3].

MATERIALS AND METHODS

The cyclodextrinyl derivatives were previously produced and characterized [11]. Triphenylphosphine was from Aldrich. The Kryptofix K22 crown ether was provided by Merck. The carbon dioxide from Messer exhibited a purity of 99.995%. The dichloromethane and methanol used were HPLC grade from VWR Prolabo.

Procedure for reaction in supercritical CO₂

A determined amount of reactants was introduced into the reactor previously heated at the desired temperature (33°C). For 1 mole of K22 (**1**) crown ether, we add 2.1 equivalents of monoazido-peroacetyl- β -cyclodextrin (**4**) and 40 equivalents of triphenylphosphine. Once the reactor was sealed, CO₂ was added at tank pressure (6MPa). When the pressure is stabilized, liquefied CO₂ (3°C) was delivered to the vessel by the Lewa pump after a pre-heating up to desired pressure and temperature. This pressure is then maintained constant using an electric control system. Once the operational pressure was attained, the stirring was started. Samples (1mL) were regularly taken via the sampling system using the following protocol. By opening valve V1, sample of the fluid phase flew into the sampling loop, which was further slowly depressurized and rinsed with dichloromethane. The volume of the dichloromethane rinsing solution $V_{\text{dichloromethane}}$ was measured by pipette taking and the content in the sampling was immediately analyzed by HPLC. Samples were collected every 15 minutes but after 1h30 a sample was collected every 30 minutes. The sampling operation continued until that a constant reaction rate in the fluid phase was obtained.

HPLC Analysis

The HPLC system consists of a SpectraSystem P1000Xr quaternary pump (Finningan,USA), a SpectraSystem SCM 1000 vacuum membrane degasser, a DDL 21 evaporative light-scattering detector (Eurosep, Fr), and a Rheodyne 7125 sample injector with a constant volume loop $V_{\text{sample loop}} = 10\mu\text{l}$. Chromatograms were acquired with a WINI 10 acquisition card and recorded on a computer by using WINILAB 2 software (Perichrom, Fr). The analytical column was an unbounded silica Polaris Si-A column (150 mm*4 mm, Varian, USA); to increase the column life a Polaris Metaguard guard column (Si-A 5u, Varian, USA) was used. The thermostat used herein was a Gecko 2000 (Cluzeau,Fr) to set the column temperature at 33°C. The ELSD was set at 100°C for evaporation temperature. Compressed air was used as nebulization gas at 2 bars of working pressure. The HPLC flow rate was 1 ml/min. A binary gradient of methanol in dichloromethane was used as eluent and permit analysis in 15 minutes.

RESULTS

Experimental design and statistical analysis

We have chosen to product the compound (**3**) issued from a strategy previously applied that consist in grafting two Cyd on a bis-crown ether. Because of the poor solubility of cyclodextrins in low density scCO₂ [12], we decided to manage the experiment at the maximal working pressure of the vessel, 200 bars and thus no studying pressure effect.

Regular samplings over time and fast analysis allowed monitoring the conversion rate; when the conversion rate became constant, it was considered that the reaction was completed. Values of

conversion rate on the plateau were averaged, and the mean value was further compared to the corresponding rate issued from the analysis of the content of the reactor after releasing CO₂. The reaction was settled two times.

The reaction, assumed irreversible, can be schematically written as in (1):

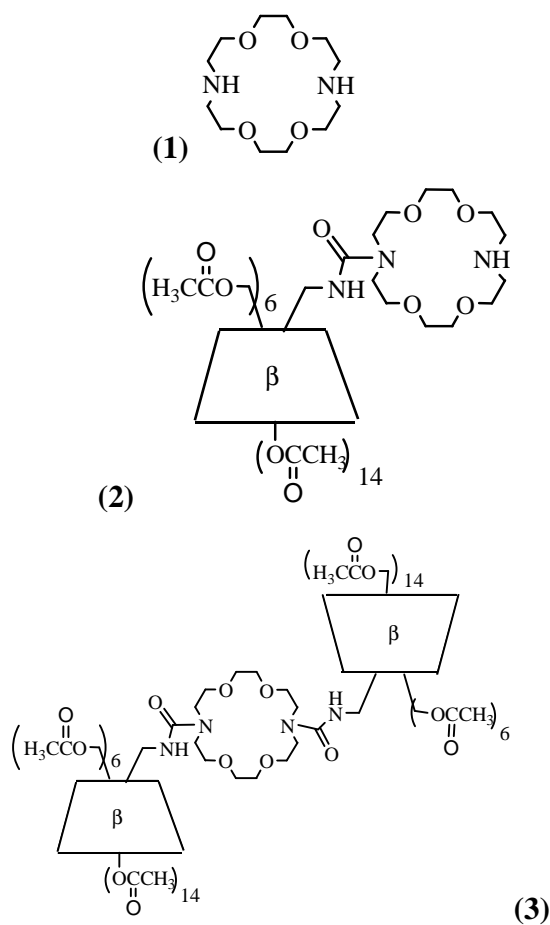
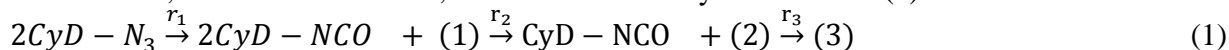


Figure 2: Crown derivatives

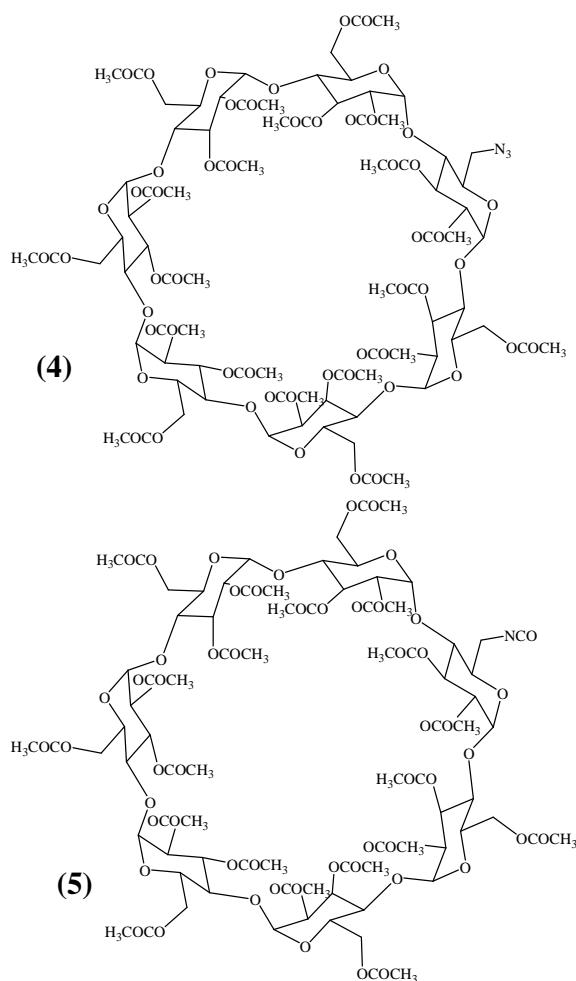


Figure 3: Cyclodextrinyls derivatives

Composition and kinetic calculations

The compositional analysis was performed using HPLC-ELSD because this method offers a relatively fast separation of the reaction mixture into P ϕ 3, azide, isocyanate and crown derivatives by high performance liquid chromatography (HPLC) followed by quantification by electrovaporative light-scattering detector (ELSD). Such detection method is reliable and well adapted for cyclodextrins detection [12]. We didn't try to evaluate the concentration of the

triphenylphosphine by this method, because it's used in wide excess (40eq.) and very well separated from other compounds.

Kinetic data were calculated using equation (1), assuming reaction r1, r2 and r3 follow a first order kinetic. The best values for the rate constant k_1 , k_2 , k_3 and final yield were obtained using the last squares method where the differences between the experimental and model conversion rates were minimized

Kinetic of the reaction

We can observe on figure 5 that the consumption of azide shows a first order kinetic; which is comparable with our previous results [3]. Isocyanates (5) concentrations stay at a low level, what indicates a good reactivity between isocyanates and -NH function of the K22 crown ether.

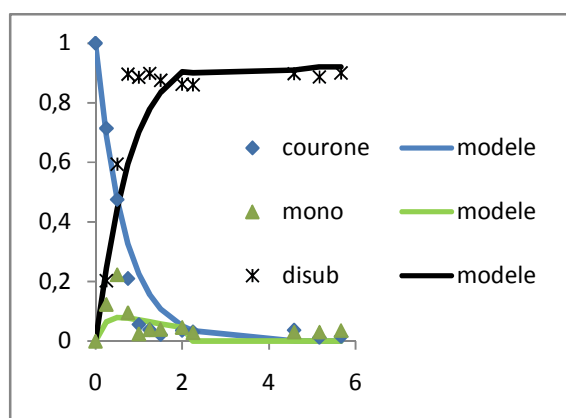


Figure 4: Crown derivatives evolution

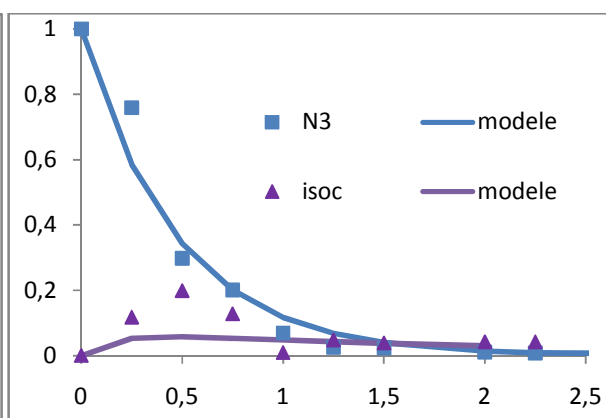


Figure 5: CyD derivatives evolution

Figure 4 shows that K22 (1) is rapidly consumed to product mono substituted crown (2). The final product (3) appears soon and the quantity of (2) is low, what indicates that there is a good reactivity between isocyanates (5) and the -NH function, even when the crown is one time substituted.

Compound (3) was usually product in DMF in 24 hours with yield of 42% [4]. The same synthesis in CO₂ is widely faster and moreover permits better yield, what indicates the usefulness of CO₂ as solvent and reactant for such synthesis.

CONCLUSION

The use of supercritical carbon dioxide as media for this reaction shows a good efficiency with yield above 92% and reaction in less than 2 hours. Moreover, the use of CO₂ avoids the time-lasting solvent purification and de-watering step due to the highly hydrophilic isocyanates intermediates. As expected, isocyanates shows good reactivity in scCO₂ with -NH functions of the K22 crown, and the reactivity of one -NH function seems not to be affected by the functionalization of the other group.

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