

SUPPORTING INFORMATION

Complexation of Sesquiterpene Lactones with Cyclodextrins: Synthesis and Effects on their Activities on Parasitic Weeds.

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Table of Contents

- S3. Method for the etiolated wheat coleoptile bioassay.**
- S4. Method for the STS bioassay.**
- S6. Method for the parasitic weed bioassay.**
- S.8 FTIR-ATR spectra of kneading and coprecipitation complexes.**
- S.15. ^1H NMR spectra of coprecipitation complexes.**
- S18. Geometry optimizations for less stable complexes and separated compounds.**

Method for the etiolated wheat coleoptile bioassay.

Etiolated wheat coleoptiles. Wheat seeds (*Triticum aestivum* L. cv. Catervo) were sown in water moistened 15 cm diameter Petri dishes and grown in the dark at 25 ± 1 °C for 4 days. Under green safelight, the shoots were removed from their roots and caryopses and placed in a van der Weij guillotine to cut and discard the apical 2 mm. The next 4 mm were taken and used for bioassays. Compounds **1**, **2** and **3** were predissolved in DMSO (0.5%) and diluted in phosphate-citrate buffer containing 2% sucrose at pH 5.6 to the final bioassay concentrations (1000, 300, 100, 30 and 10 μ M).

In addition, negative controls were used. They were two buffered aqueous solutions without any test compound and with DMSO (control for compounds **1**, **2** and **3**) or without it (control for cyclodextrins and their complexes).

Each test tube containing 5 coleoptiles and 2 mL of solution was replicated three times and was put in a roller tube apparatus at 0.25 rpm and 25 °C in the dark for 24 hours. The coleoptiles were measured by digitalization of their images. Data were statistically analysed using Welch's test and are presented as percentage differences from control. Thus, zero represents the control, positive values stimulation of growth and negative inhibition.

Method for the STS bioassay.

The STS selected were monocotyledon onion (*Allium cepa* L.) and the dicotyledons tomato (*Lycopersicon esculentum* Mill.), cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.). These seeds were supplied by 'Fitó Seeds' (Barcelona, Spain).

Twenty seeds were put in Petri dishes (50 mm diameter) containing one sheet of 'Whatman No.1' filter paper as a support. Germination and growth were conducted in aqueous solutions at controlled pH by using 10^{-2} M 2-[*N*-morpholino]ethanesulfonic acid (MES) and 1 M NaOH (pH 6.0). The concentrations tested were the same as the coleoptile bioassay and two negative controls were used, the buffer with and without 0.5 % DMSO. The commercial herbicide Logran[®], whose original formulation is a combination of *N*²-*tert*-butyl-*N*⁴-ethyl-6-(methylsulfanyl)-1,3,5-triazine-2,4-diamine (terbutryn, 59.4%) and 2-(2-chloroethoxy)-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)carbamoyl]benzene-1-sulfonamide (triasulfuron, 0.6%), was used as a positive control at the same concentrations as the other compounds.

Compound **1** was predissolved in 0.5% DMSO. Four replicates were done for each dilution and control. To each Petri dish was added 1 mL of treatment or control and sealed with Parafilm to ensure close-system models. Seeds were further incubated in a Memmert ICE 700 controlled environment growth chamber at 25 °C in the dark. Each seed was incubated a different time: 4 days for cress, 5 days for tomato, 6 days for lettuce and 7 days for onion. After the growth period, the plants were frozen at -10 °C for 24 h in order to avoid subsequent growth during the measurement process.

Evaluated parameters (germination rate, root length and shoot length) were recorded using a Fitomed[©] system, which allowed automatic data acquisition and statistical analysis using its associated software. Data were analyzed using Welch's test, with

significance fixed at 0.01 and 0.05. The results are presented as percentage differences from the control. Zero values represent the control, positive values stimulation and negative values inhibition. The statistical significance is expressed with letters above the graph bars, 'a' meaning significantly different from control with 0.01 confidence and 'b' meaning different from control with a confidence from 0.01 to 0.05. The absence of a letter indicates no significant difference from control values.

Method for the parasitic weed bioassay.

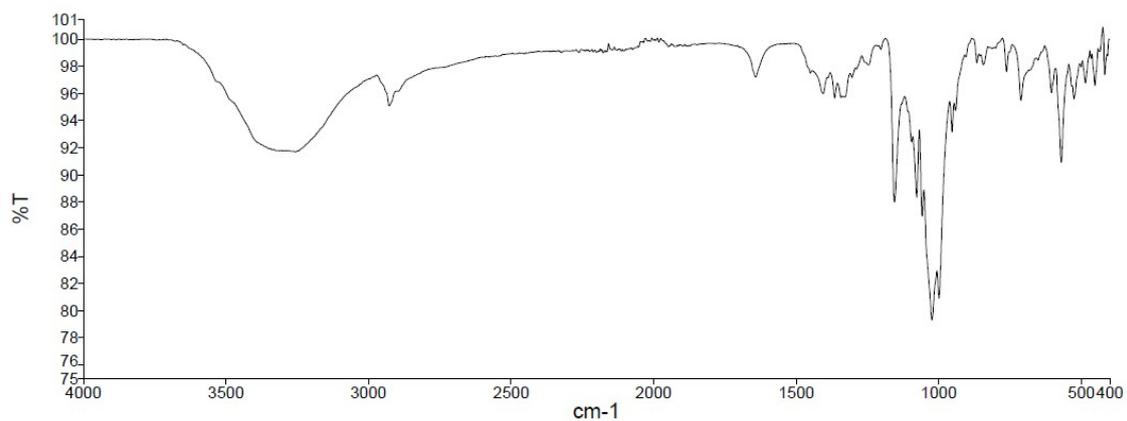
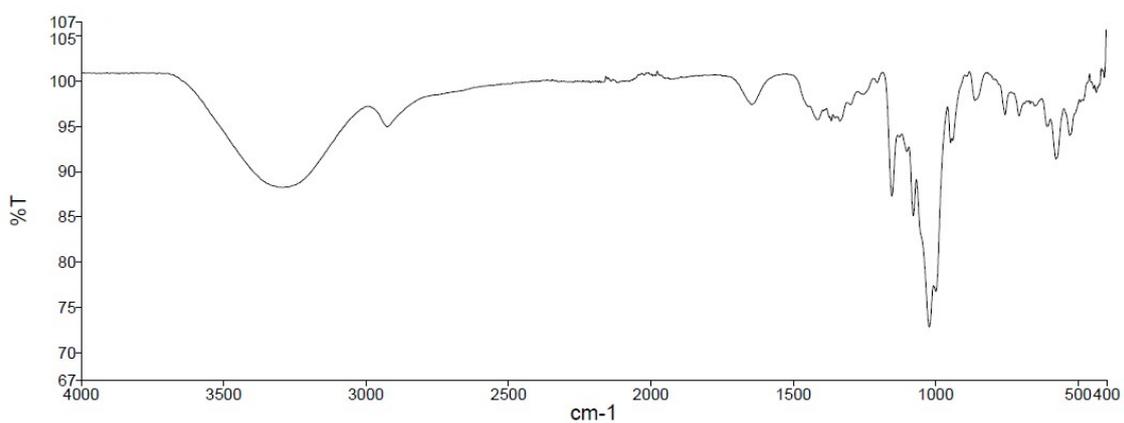
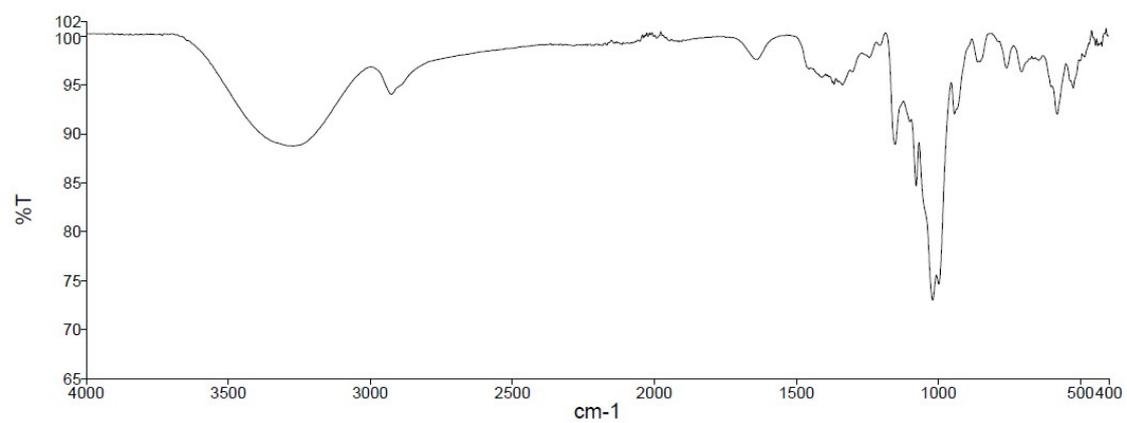
Compounds **1**, **2**, **3**, β -CD, γ -CD, and their complexes synthesized by coprecipitation were tested on the seeds of five parasitic weed species: *Orobanche cumana*, *Orobanche minor*, *Phelipanche aegyptiaca*, *Phelipanche ramosa*, and *Striga hermonthica*. The seeds were surface sterilized by immersion in 0.5% (w/v) NaOCl and 0.02% (v/v) Tween 20 with sonication for 2 min. The samples were rinsed thoroughly with sterile distilled water and dried in a laminar air flow cabinet.

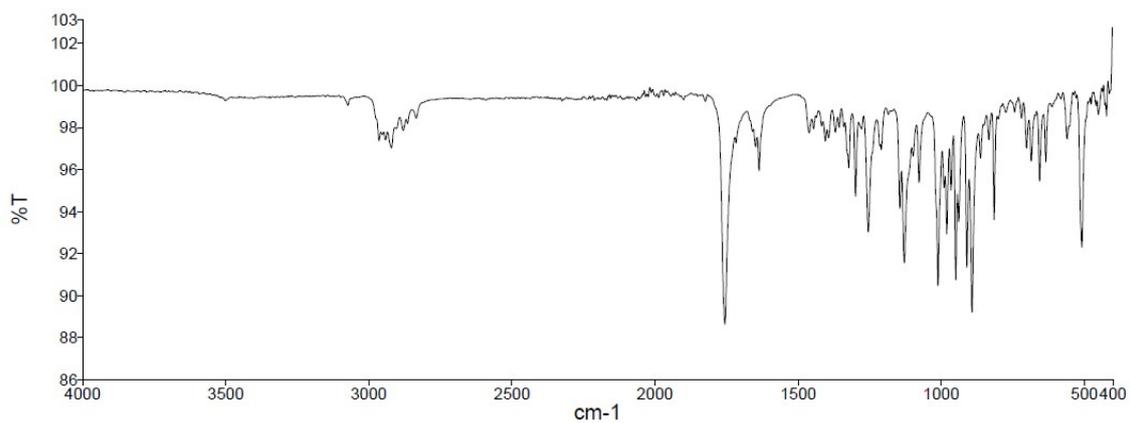
Seeds of obligated parasitic weeds require a period of conditioning in order to become sensitive to germination-inducing factors. The length of the period and temperature required varies between *Orobanche* and *Striga* species. To promote conditioning, approximately 100 seeds of each parasitic weed species were placed separately on 9 mm diameter glass fiber paper disks (GFFP) moistened with 50 μ L of sterile distilled water and placed in 10 cm sterile Petri dishes in the dark at 21 °C during 10 days for *Orobanche* and *Phelipanche* species and at 30 °C during 14 days for *Striga* species.

After seed conditioning, GFFP disks were transferred in a laminar flow cabinet on to sterile filter paper to remove excess of water and then they were transferred to a new 10 cm sterile Petri dish. The test samples **1**, **2** and **3** were dissolved in acetone and diluted with sterilized MQ water to a concentration range of 100 to 0.1 μ M. The final concentration of acetone was adjusted to 1% (v/v). In the case of the samples containing cyclodextrins, acetone was not necessary and not used. Disks containing conditioned seeds were treated with a 50 μ L aliquot of the respective test solution. Each treatment was replicated 3 times. Two controls were introduced for comparison, a negative control of seeds treated with MQ water (containing 1% acetone) and a positive control of the synthetic strigolactone **GR24** at the same concentration range of the tested compounds.

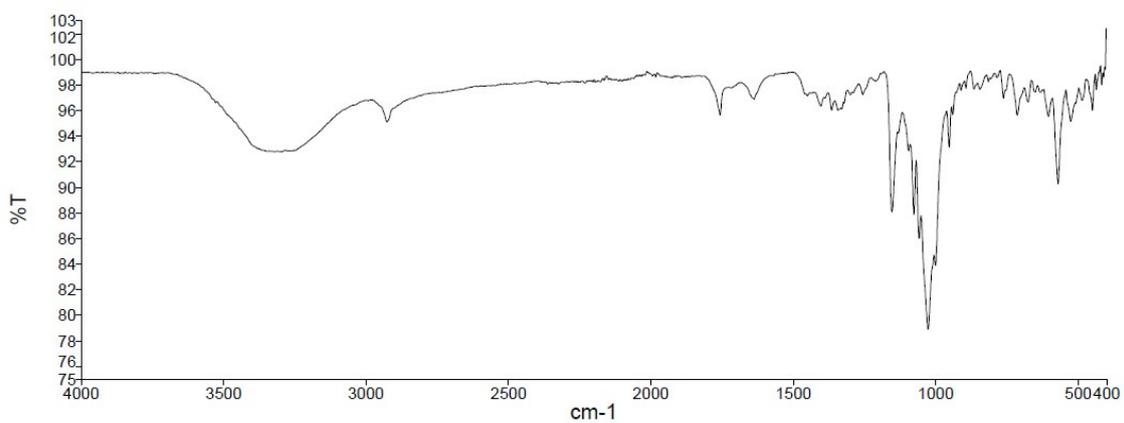
The seeds were incubated in the dark at 21 °C for 7 days for *Orobanche* and *Phelipanche* species and 4 days at 30 °C for *Striga* species prior to examination for germination. Seeds with an emerged radicle through the seed coat were scored as germinated, as observed using a stereoscopic microscope at 30× magnification, and the percentage of germination was established for each dish.

The bioassays were performed twice with at least 3 replicates. Percentage data were approximated to a normal frequency distribution by means of angular transformation ($180/\pi \times \arcsine(\sqrt{\%/100})$) and subjected to analysis of variance (ANOVA) using SPSS software for Windows, version 21.0 (SPSS Inc., Chicago, Illinois, USA). The significance of mean differences between each treatment against negative control was evaluated by the two-sided Dunnett's test. Null hypothesis was rejected at the level of 0.05.

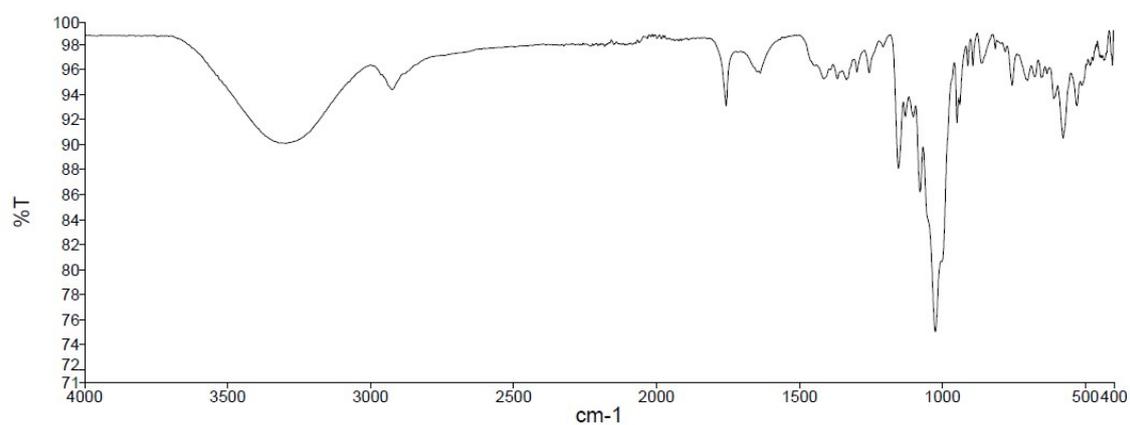
FTIR-ATR spectra of kneading and coprecipitation complexes.**FTIR-ATR spectrum of α -cyclodextrin.****FTIR-ATR spectrum of β -cyclodextrin.****FTIR-ATR spectrum of γ -cyclodextrin.**



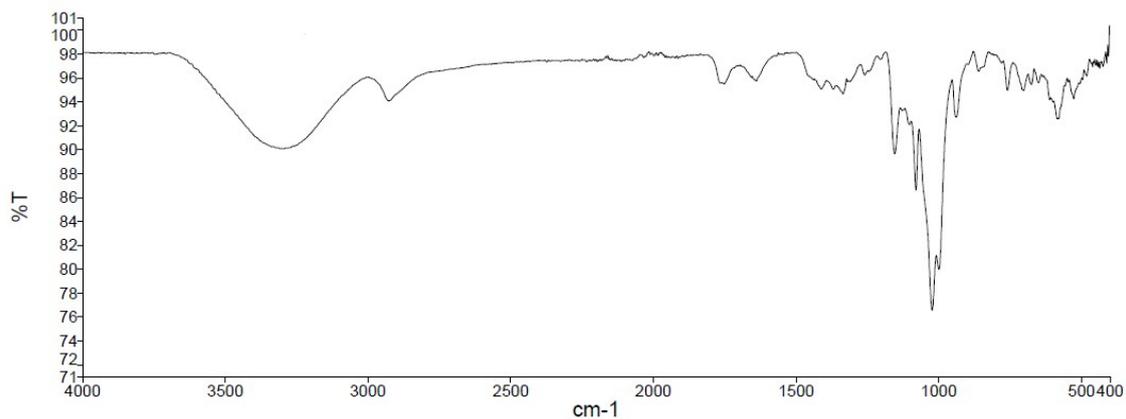
FTIR-ATR spectrum of 1.



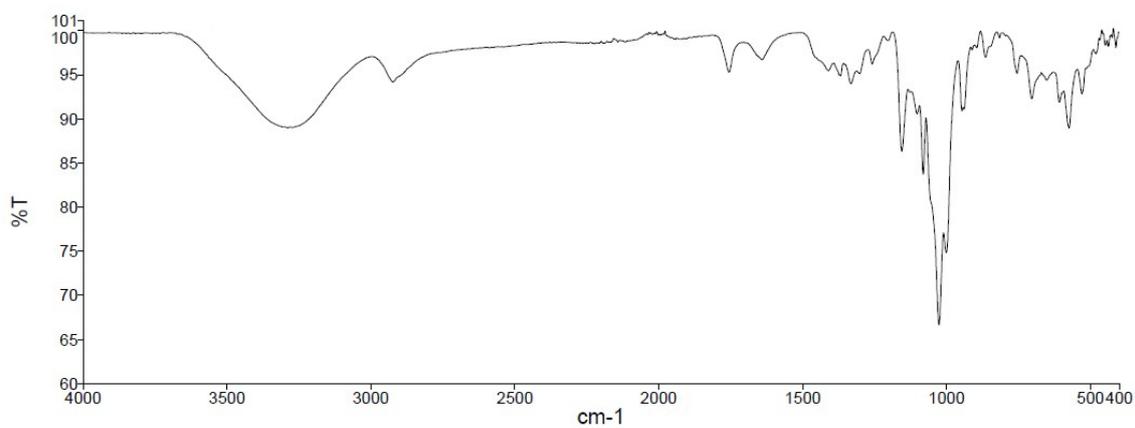
FTIR-ATR spectrum of 1- α -cyclodextrin (kneading method).



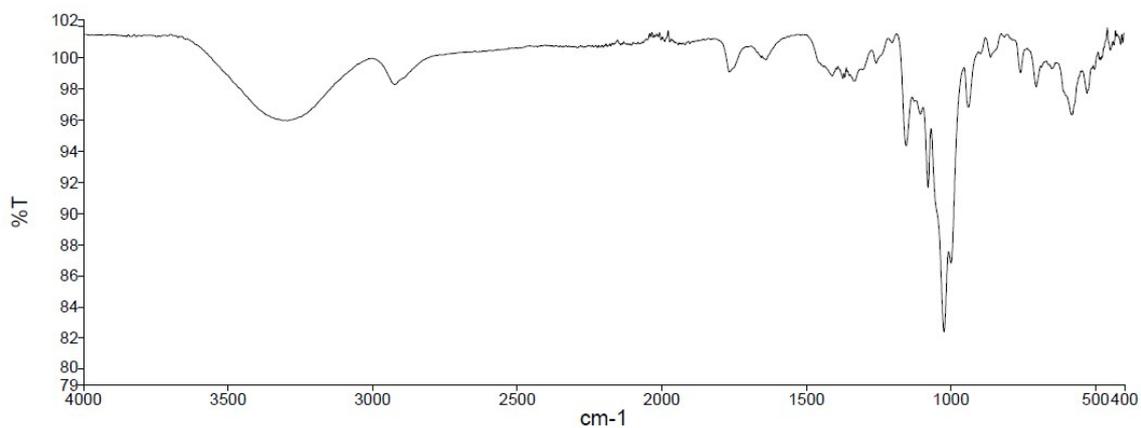
FTIR-ATR spectrum of 1- β -cyclodextrin (kneading method).



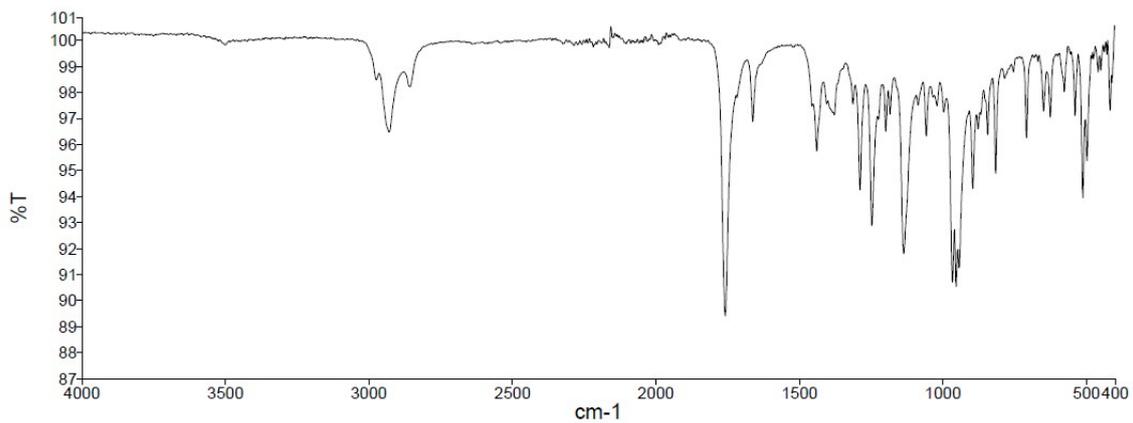
FTIR-ATR spectrum of 1- γ -cyclodextrin (kneading method).



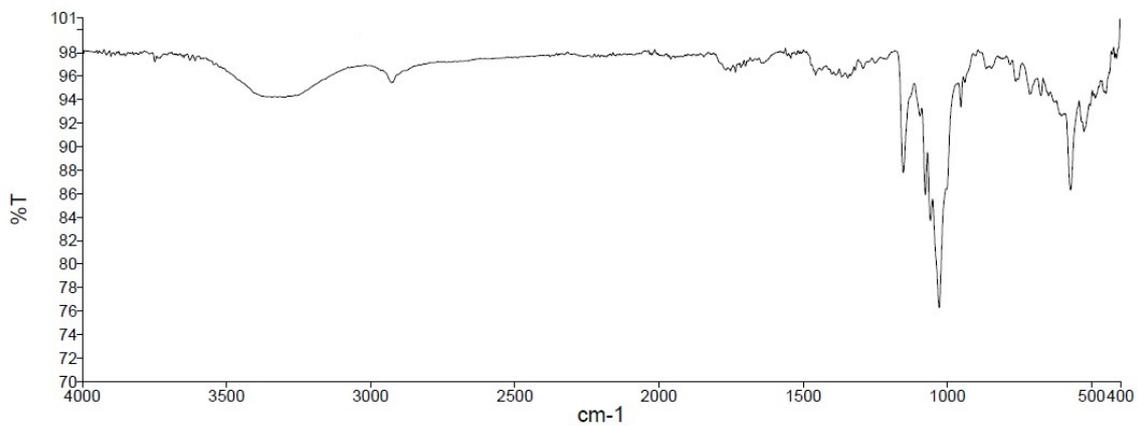
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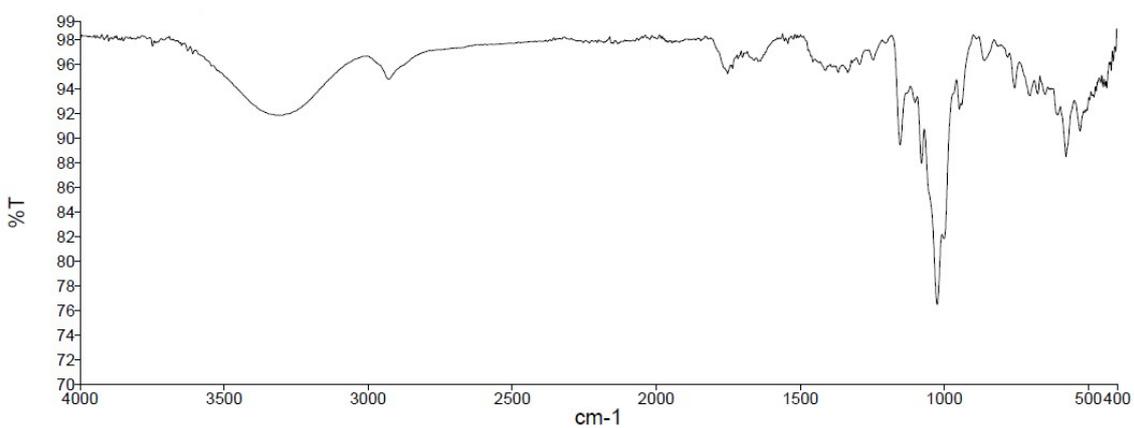
FTIR-ATR spectrum of 1- γ -cyclodextrin (coprecipitation method).



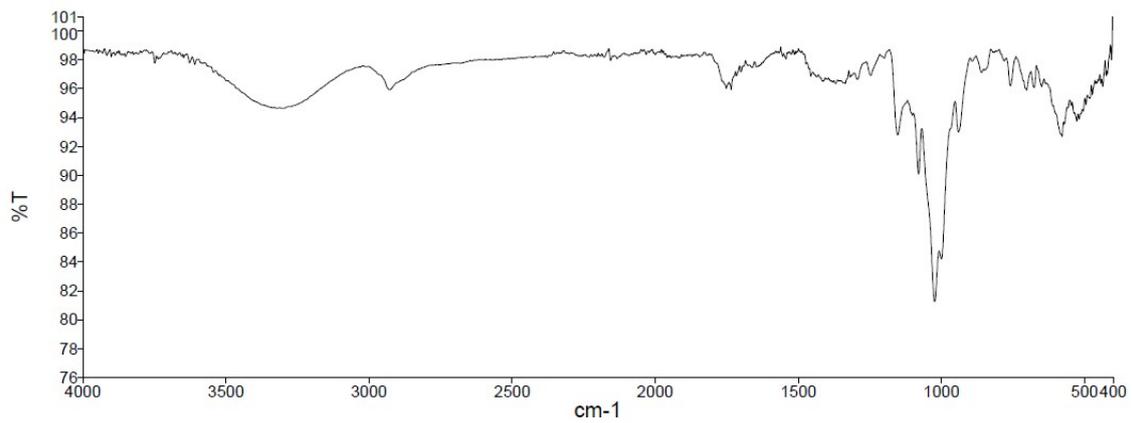
FTIR-ATR spectrum of 2.



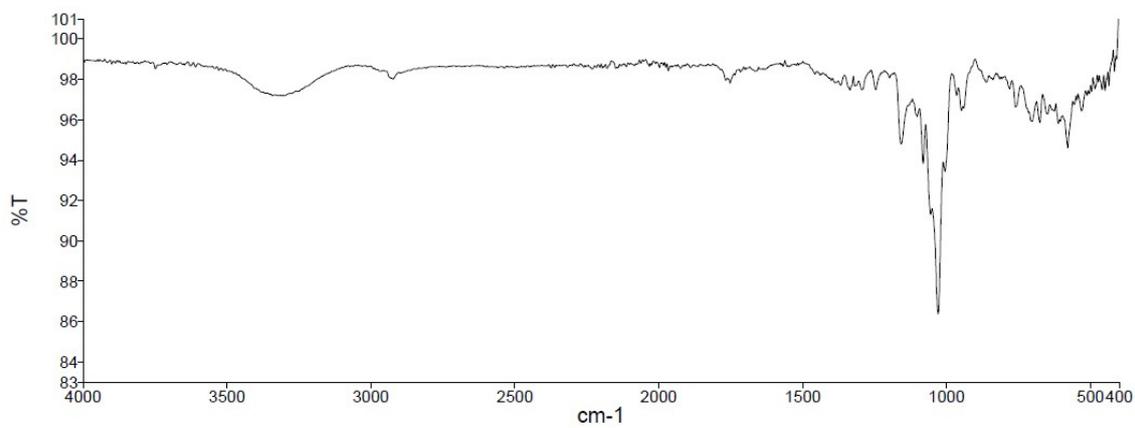
FTIR-ATR spectrum of 2- α -cyclodextrin (kneading method).



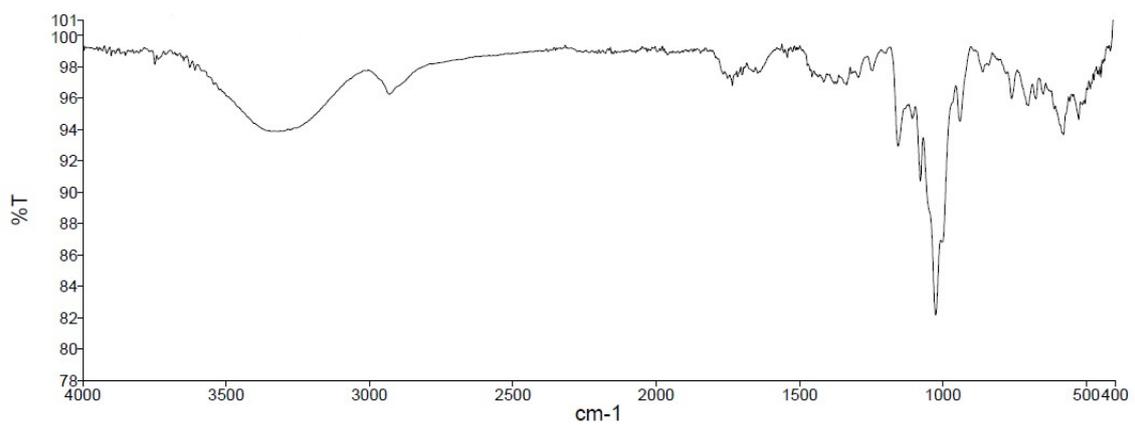
FTIR-ATR spectrum of 2- β -cyclodextrin (kneading method).



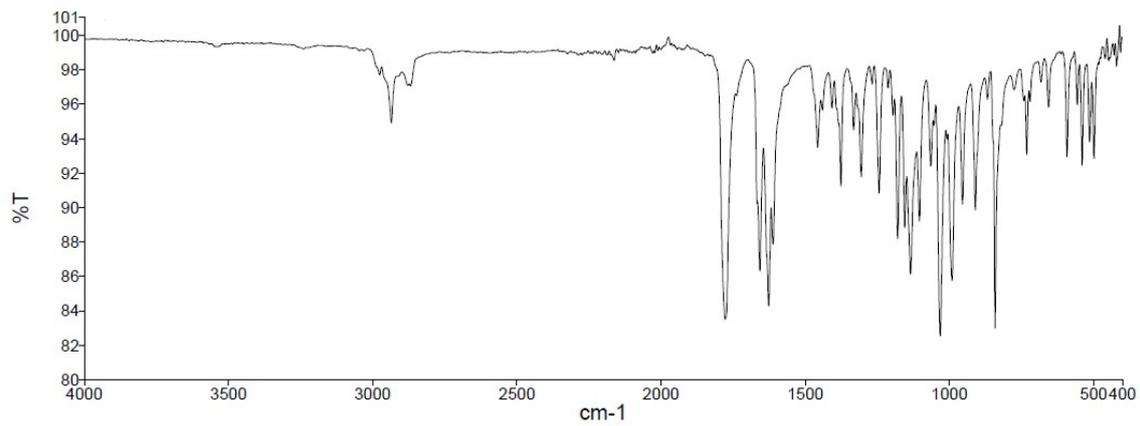
FTIR-ATR spectrum of 2- γ -cyclodextrin (kneading method).



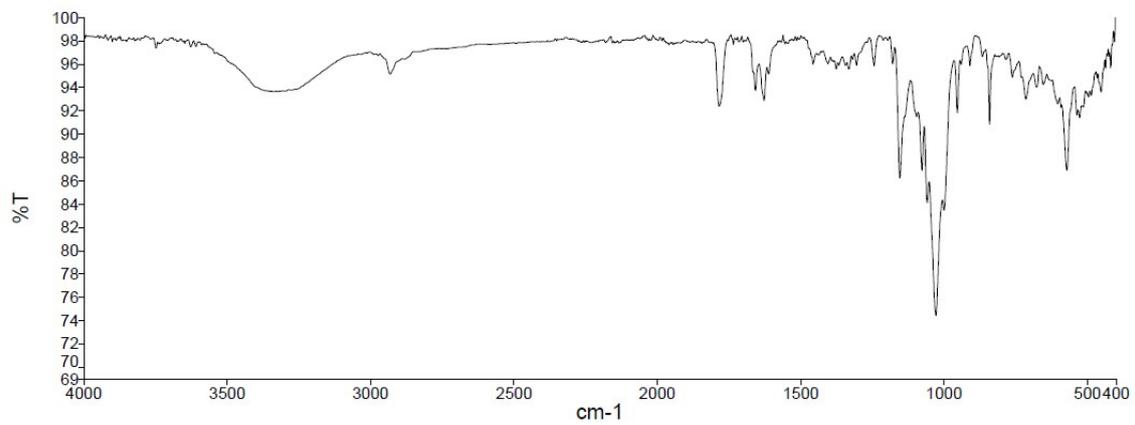
FTIR-ATR spectrum of 2- β -cyclodextrin (coprecipitation method).



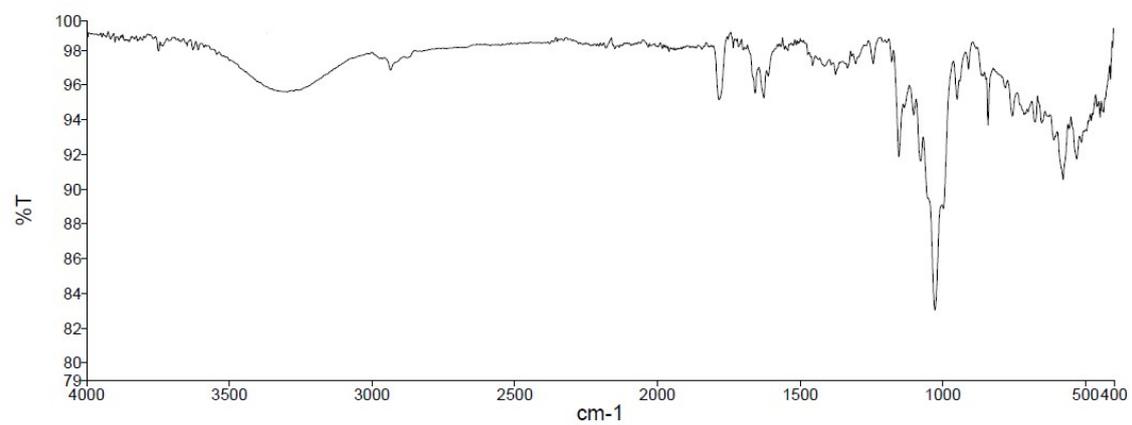
FTIR-ATR spectrum of 2- γ -cyclodextrin (coprecipitation method).



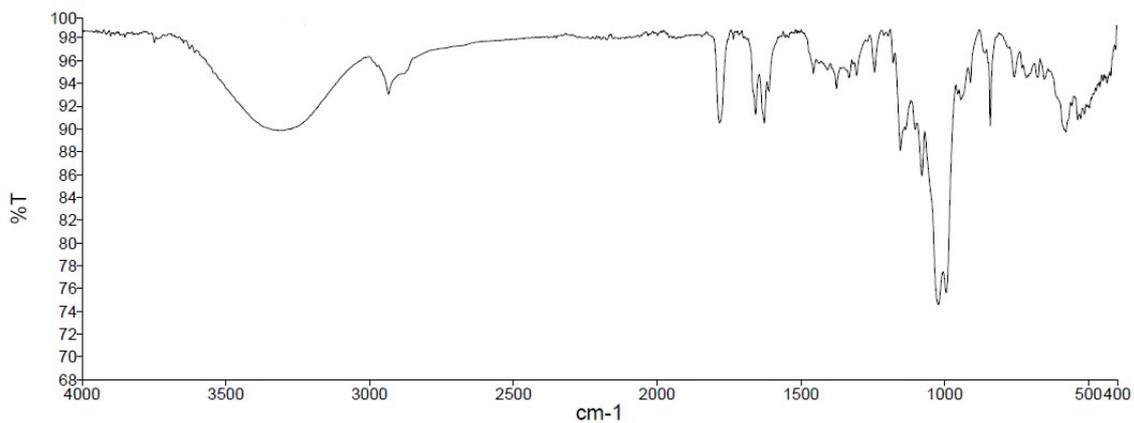
FTIR-ATR spectrum of 3.



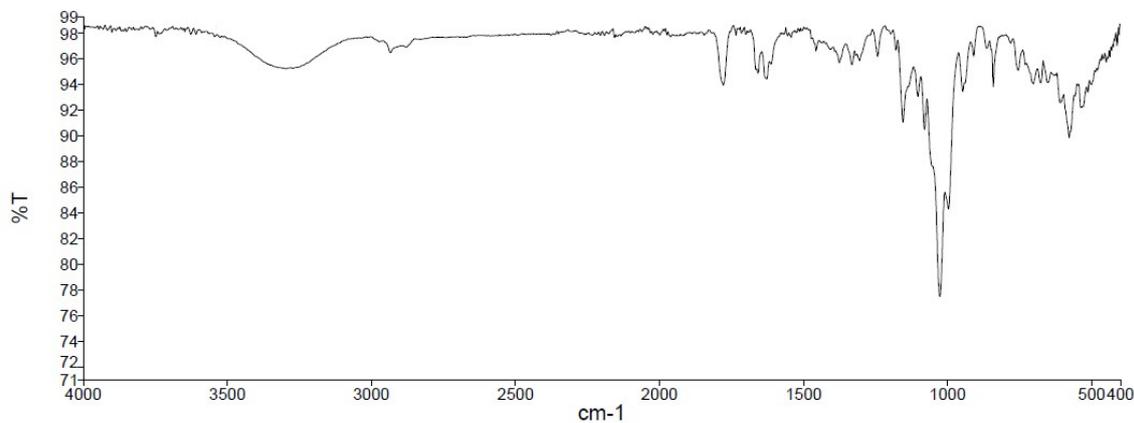
FTIR-ATR spectrum of 3- α -cyclodextrin (kneading method).



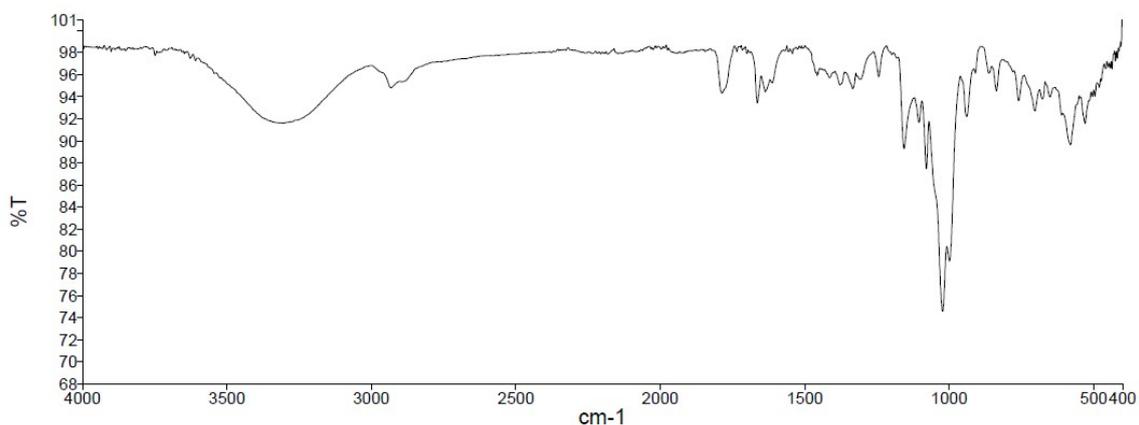
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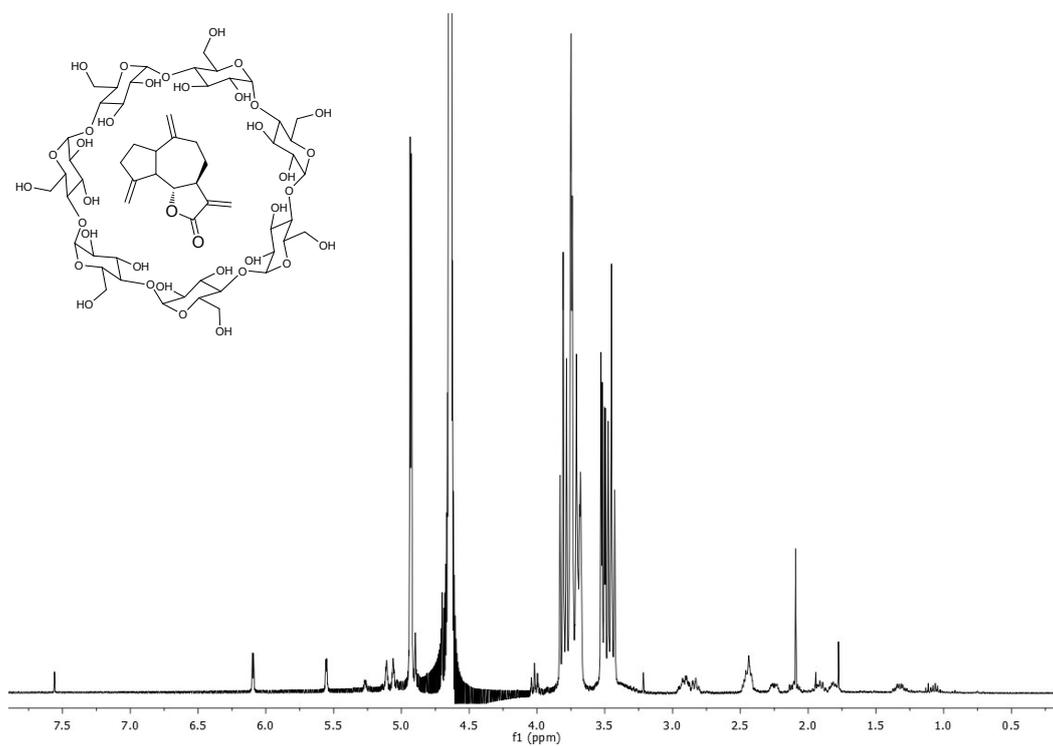
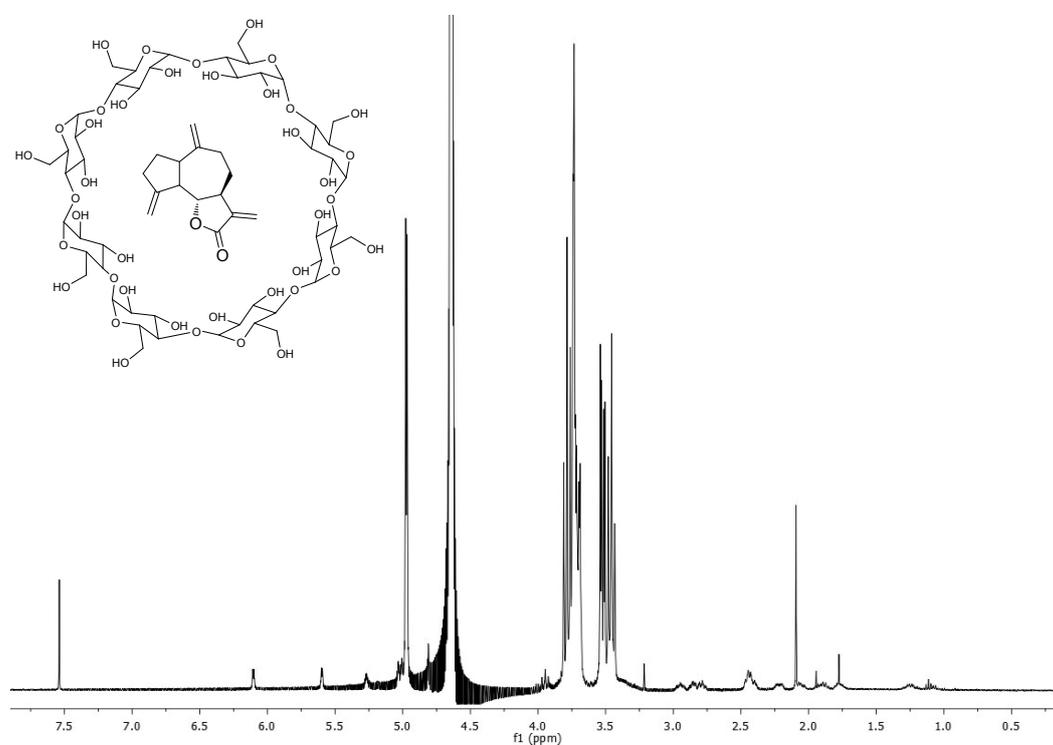
FTIR-ATR spectrum of 3- γ -cyclodextrin (kneading method).

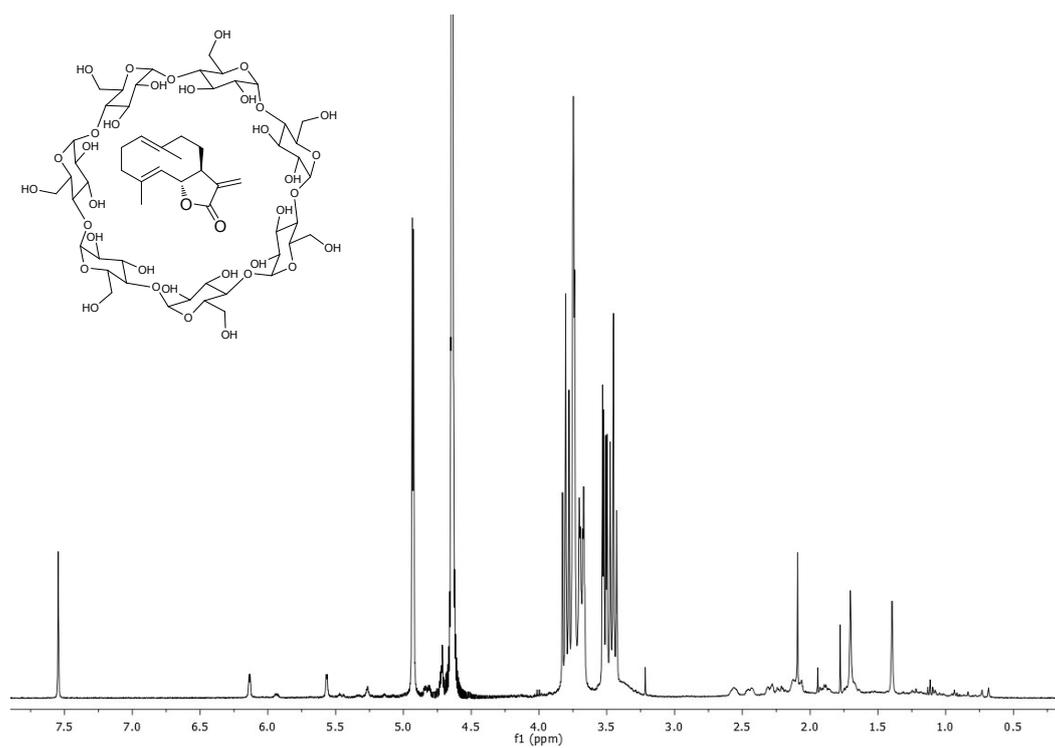


FTIR-ATR spectrum of 3- β -cyclodextrin (coprecipitation method).

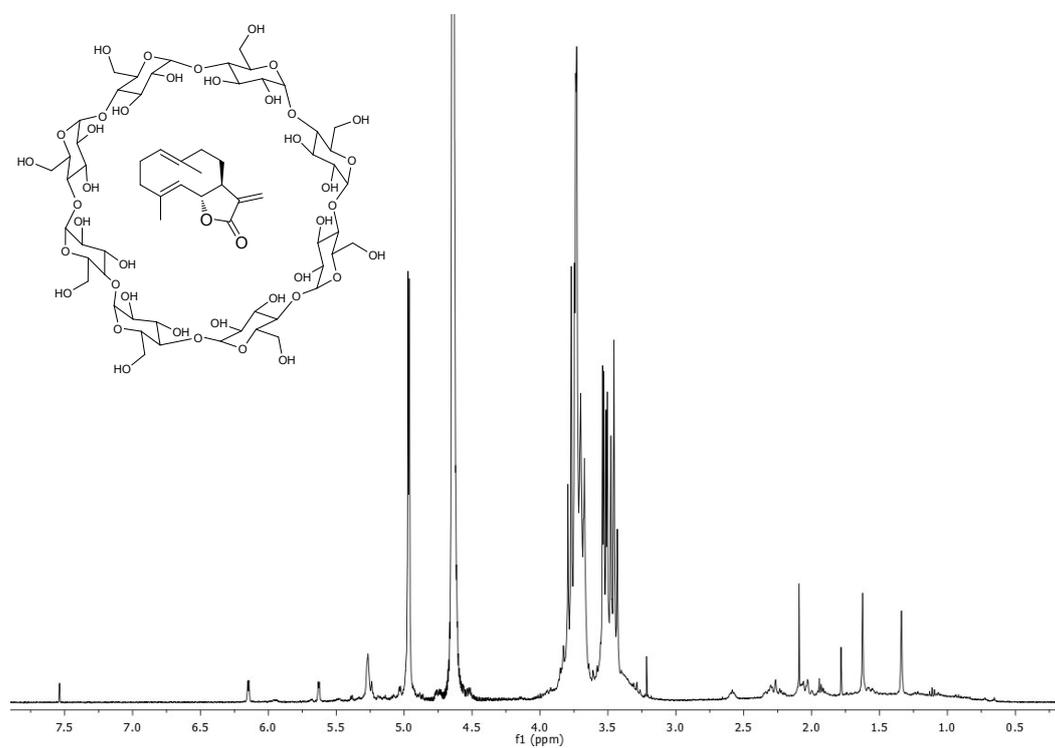


FTIR-ATR spectrum of 3- γ -cyclodextrin (coprecipitation method).

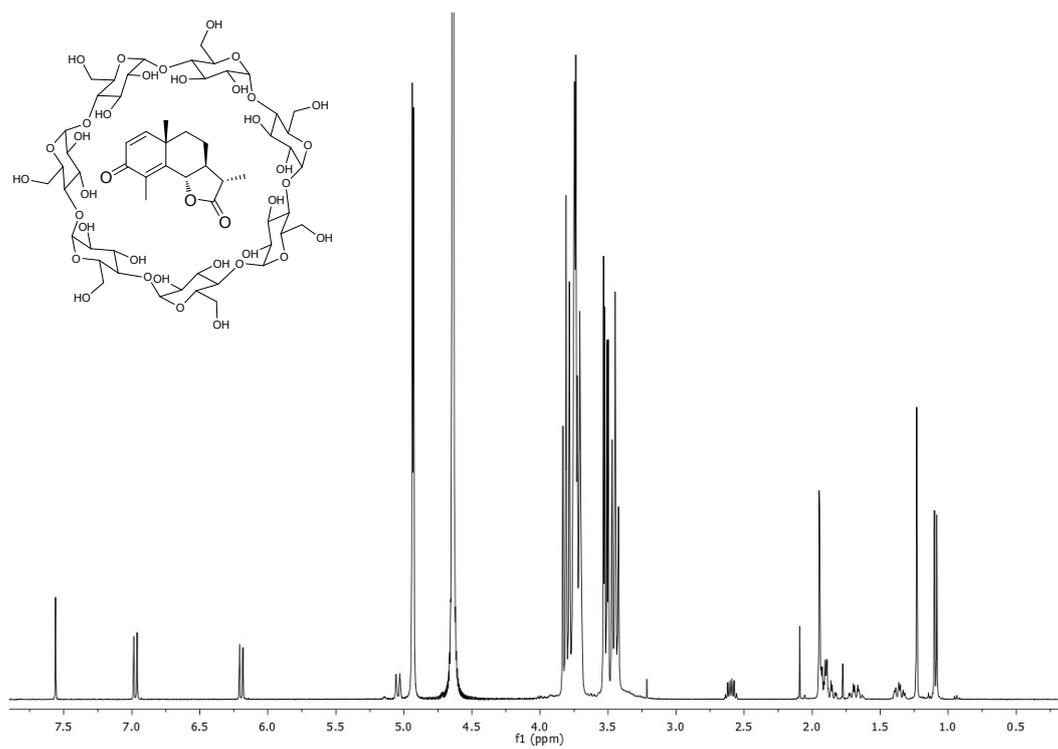
^1H NMR spectra of coprecipitation complexes. **^1H NMR spectrum of 1- β -cyclodextrin (coprecipitation method).** **^1H NMR spectrum of 1- γ -cyclodextrin (coprecipitation method).**



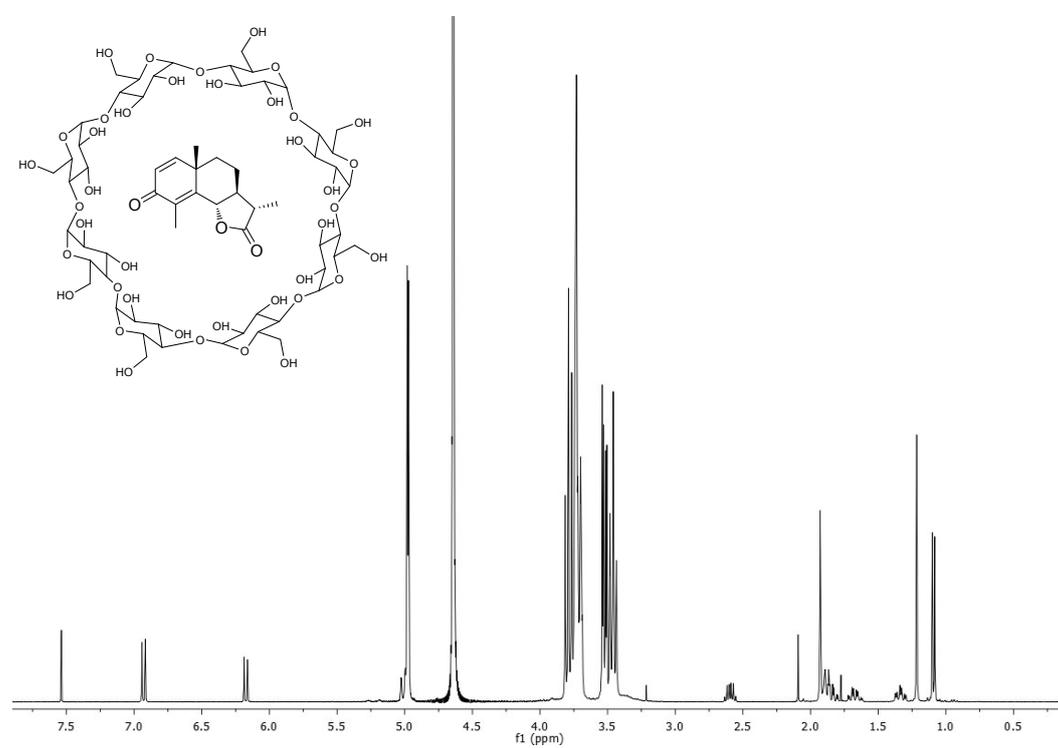
¹H NMR spectrum of 2-β-cyclodextrin (coprecipitation method).



¹H NMR spectrum of 2-γ-cyclodextrin (coprecipitation method).

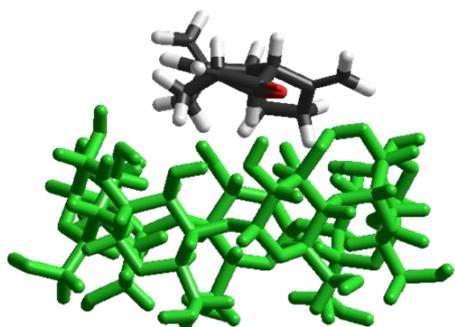


¹H NMR spectrum of 3-β-cyclodextrin (coprecipitation method).

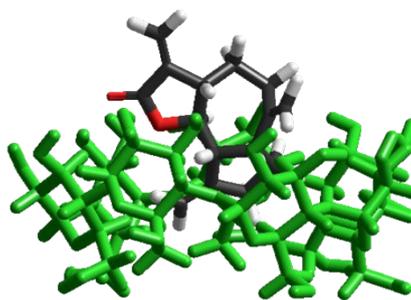


¹H NMR spectrum of 3-γ-cyclodextrin (coprecipitation method).

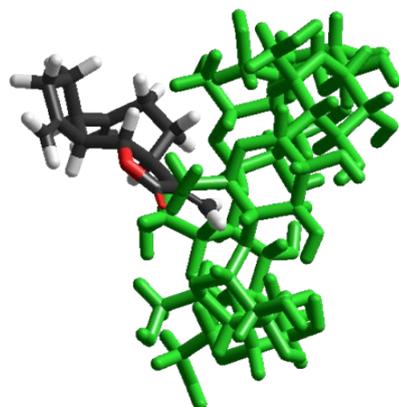
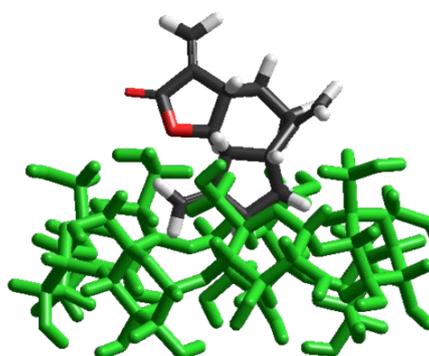
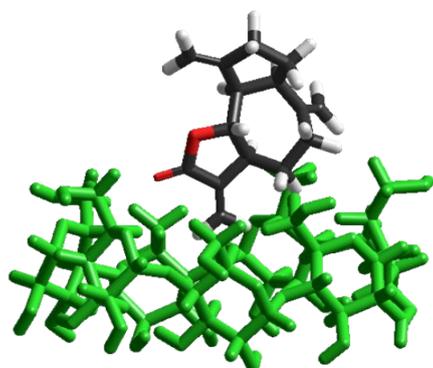
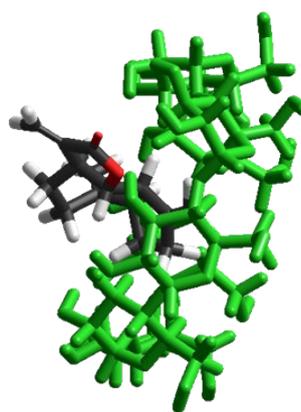
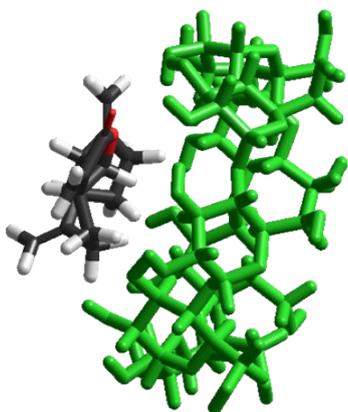
Geometry optimizations for less stable complexes and separated compounds.



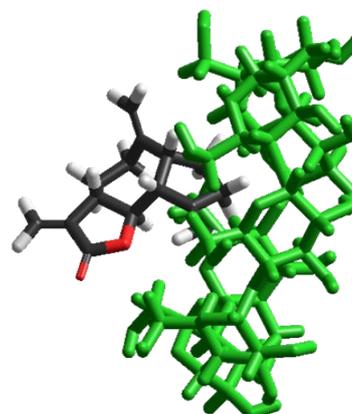
Separated 1 and β -CD



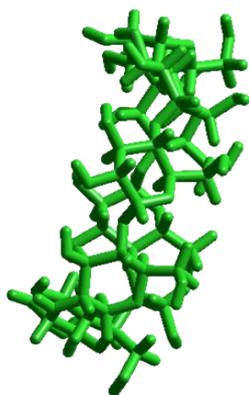
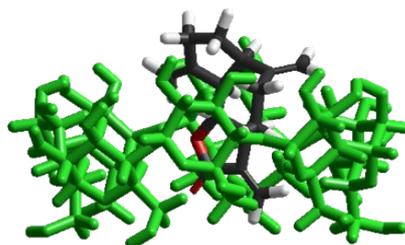
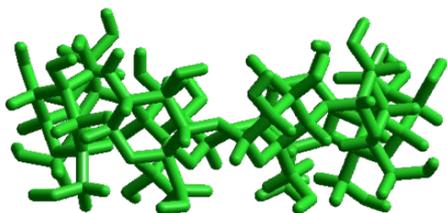
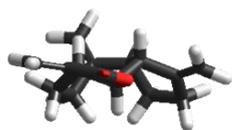
1- β -CD A



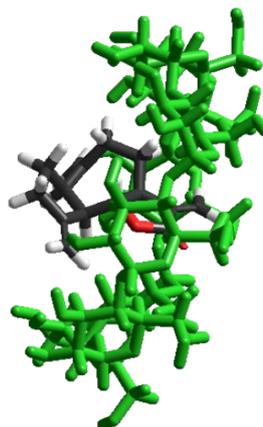
1- β -CD C



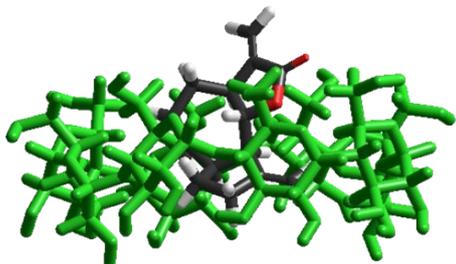
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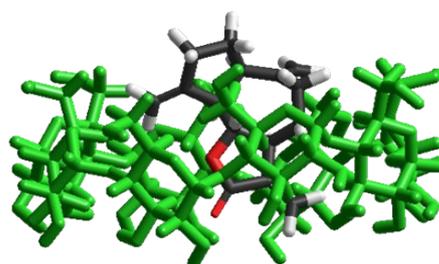
Separated **1** and γ -CD



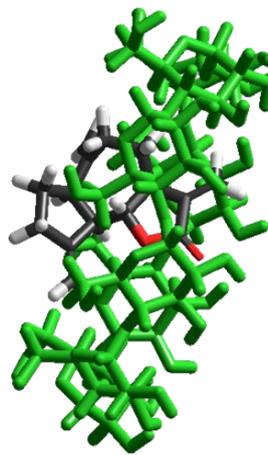
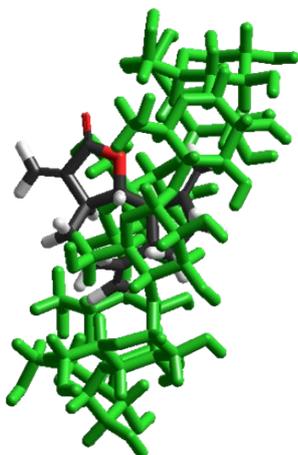
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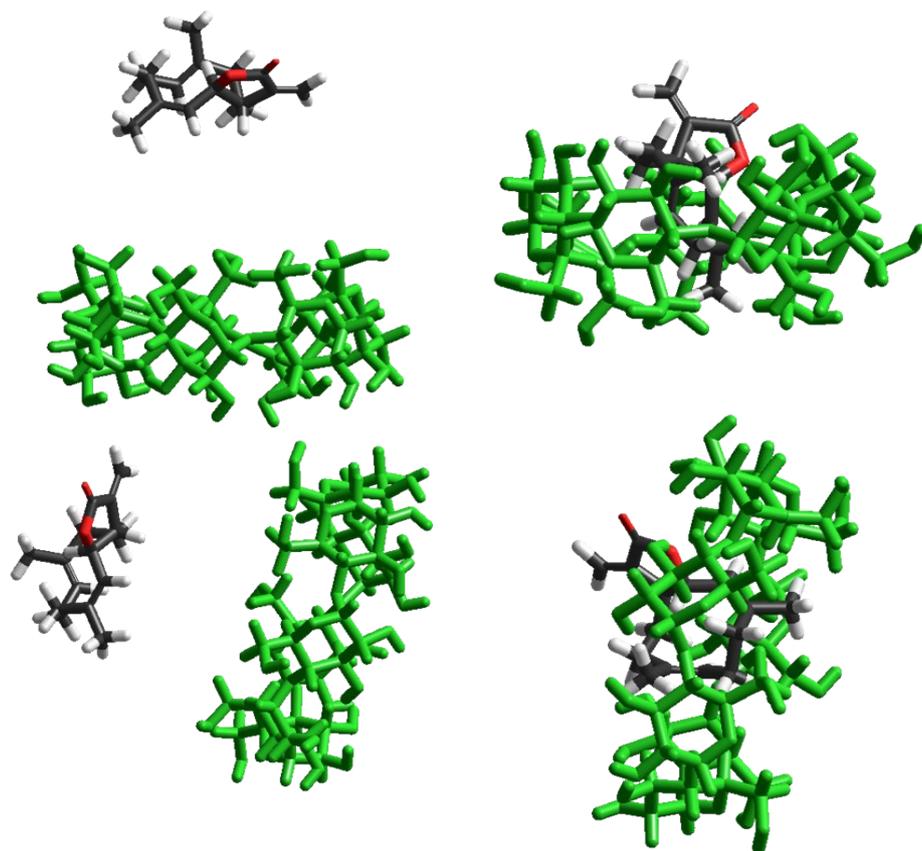


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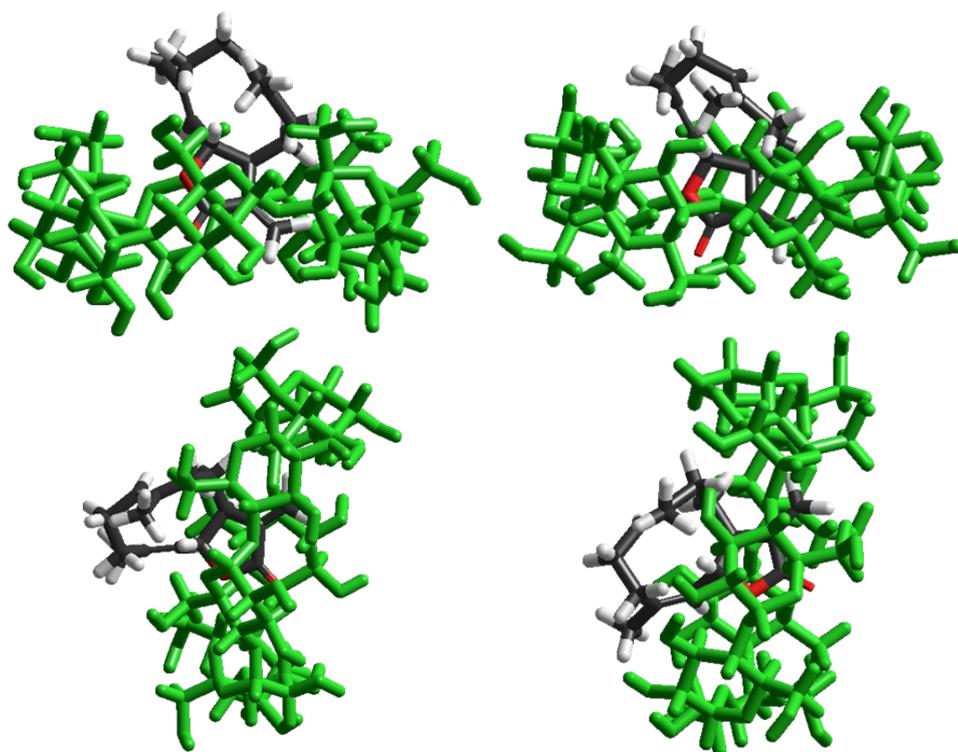
1- γ -CD **C**





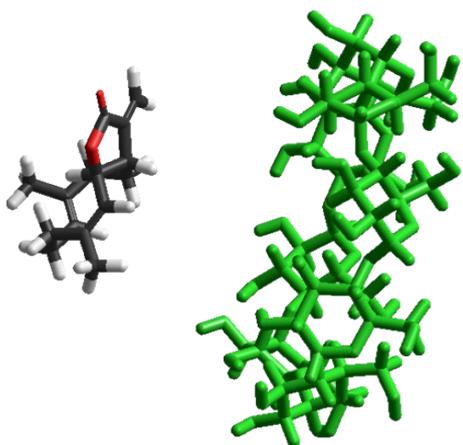
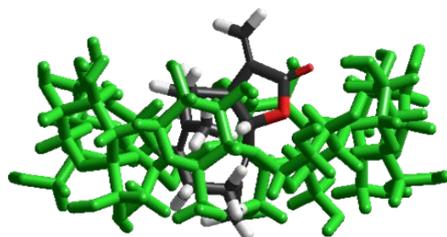
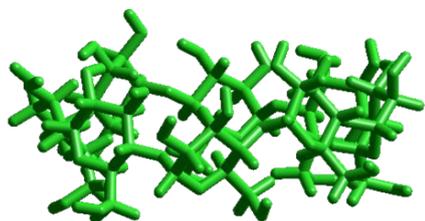
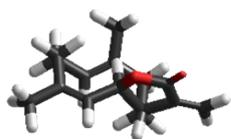
Separated 2 and β -CD

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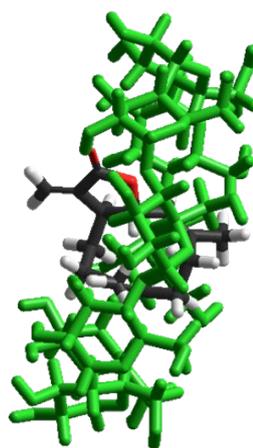


2- β -CD C

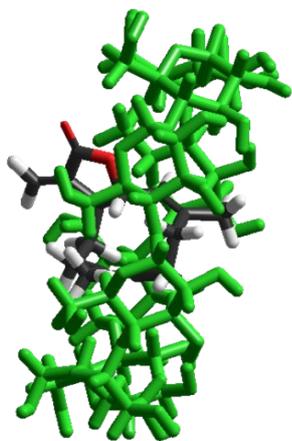
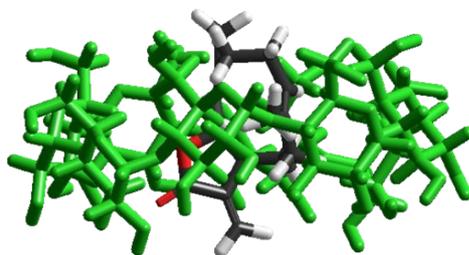
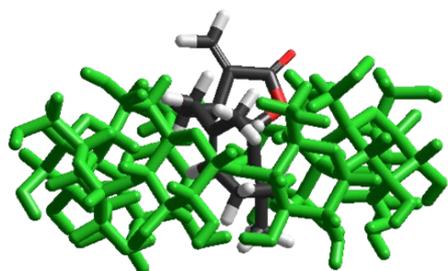
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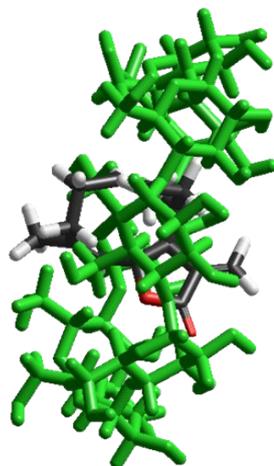
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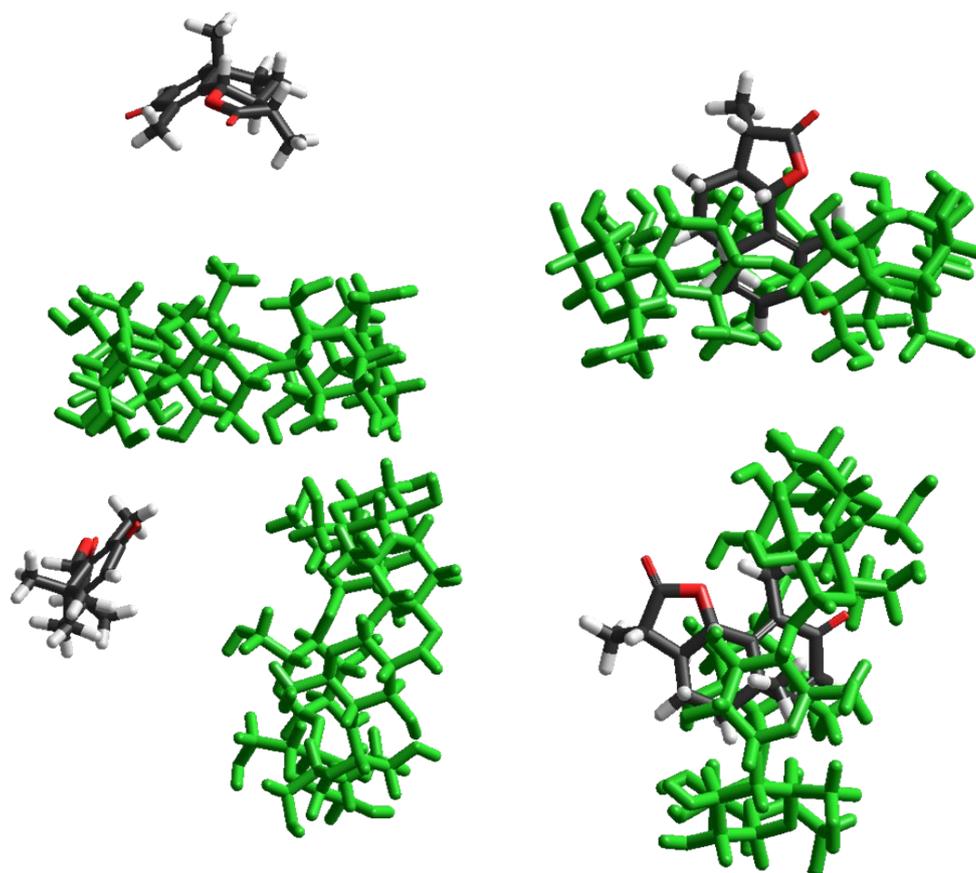
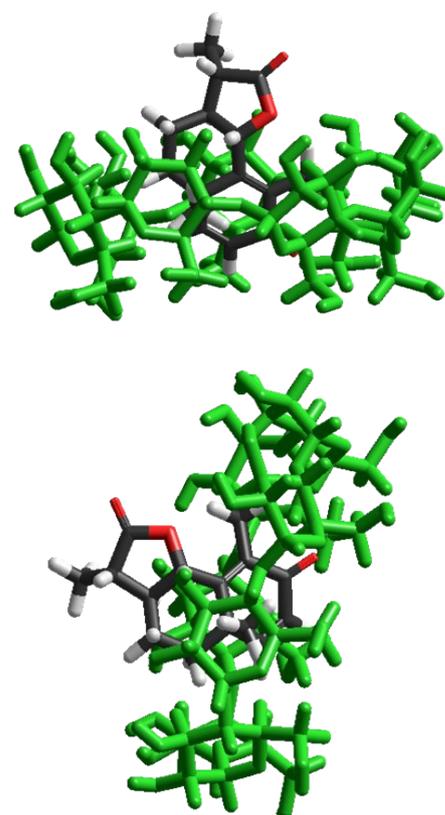
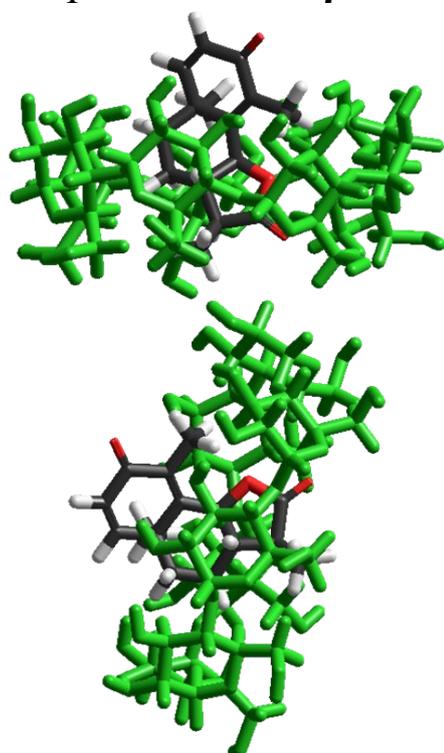
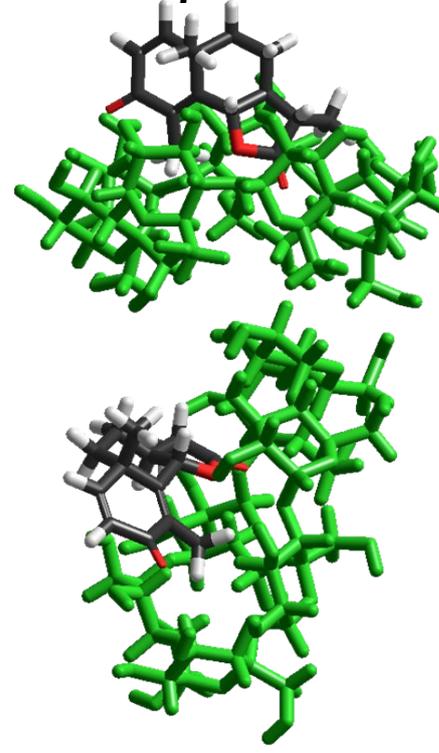
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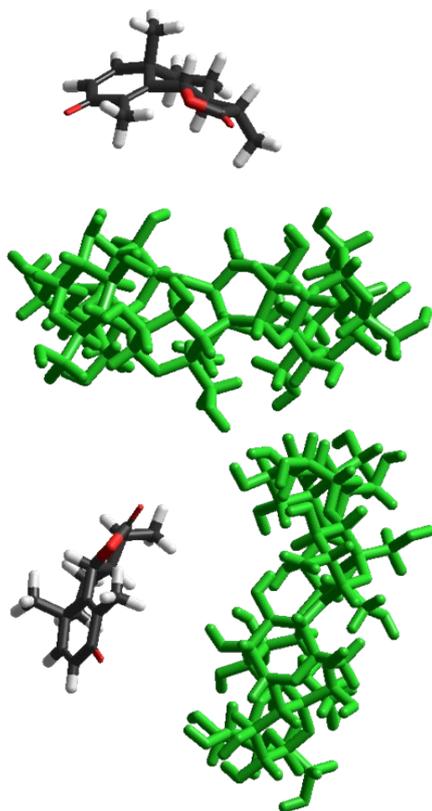


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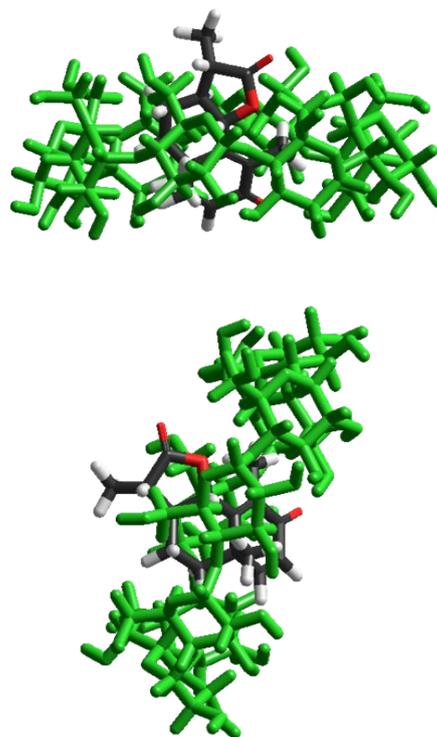


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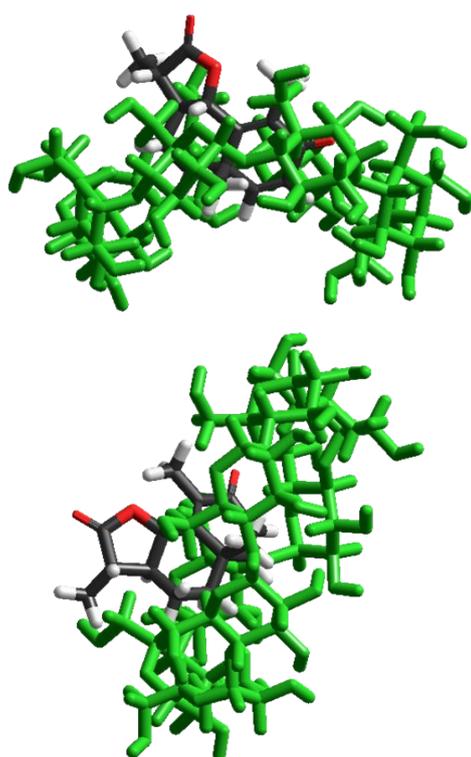
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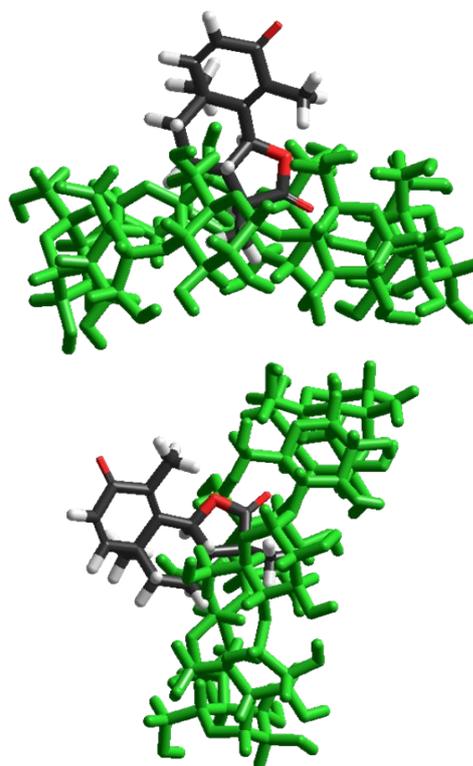
Separated 3 and γ -CD



3- γ -CD B



3- γ -CD C



3- γ -CD D