Catalytic Ship-In-A-Bottle Assembly within Hollow Porous Nanocapusles

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Chemicals. 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was purchased from Avanti Polar Lipids, Inc. as a dry powder. Tert-butyl methacrylate (t-BMA), butyl methacrylate (BMA), used as monomers, and ethylene glycol dimethacrylate (EGDMA), used as a crosslinking agent, were purchased from Sigma-Aldrich and were passed through alumina column, to remove the inhibitor, shortly before the polymerization. Deuterated solvents (chloroform-d, benzene-d^6, D_2O, CD_3OD), p-tolyl isocyanate, 4-methoxyphenyl isocyanate, 4-tert-butylphenyl isocyanate, paraformaldehyde, sodium azide and photoinitiator (2,2-dimethoxy-2-phenyl-acetophenone (DPA), manufactured by Sigma-Aldrich, were used without any additional purification. 2,8,9-Triisopropyl-2,5,8,9-tetraaza-1-phosphabicyclo[3,3,3]undecane (Verkade's superbase), also from Sigma-Aldrich, was dissolved in water (pH was adjusted to 7.4). 3,5-Di-tert-butylbenzoic acid purchased from Alfa Aesar co. 1,10-diaza-18-crown-6 (DA18C6) was purchased from Acros Organics and was used as received. The solvents and other chemicals used in this study were HPLC and ACS reagent grade, respectively and were used as received.

Preparation of catalyst-loaded nanocapsules. 160 mg of DMPC was dissolved in 0.4 mL of chloroform in a test tube, then t-BMA (32 μL, 0.193 mmol), BMA (32 μL, 0.199 mmol), EGDMA (32 μL, 0.166 mmol) and initiator 2,2-dimethoxy-2-phenyl-acetophenone (3 mg, 0.01 mmol) were added. Chloroform was evaporated using a stream of purified argon to form a lipid/monomer mixture. Methylene chloride or other volatile solvents can be used instead of chloroform. The mixture was further dried in vacuum to remove traces of solvent. Eight ml of the aqueous solution of Verkade's superbase (1.5×10^{-4} M) was added to the test tube with the lipid/monomer mixture and incubated at 35 °C for 30 minutes. During the hydration of the lipid/monomer mixture, the culture tube was briefly agitated on a Vortexer every 5 min. Five freeze-thaw cycles were done to increase encapsulation of catalyst. The suspension was extruded 12 times at 35 °C through a track-etched polyester Nucleopore membrane (Sterlytech) with 0.2 μm pore size using a Lipex stainless steel extruder (Northern Lipids).

The sample was irradiated for 1.5 hours with UV light (λ = 254 nm) in a photochemical reactor (10 lamps, 32W each; the distance between the lamps and the sample was 10 cm) using a quartz tube with path length of light of approximately 3 mm.

Following the polymerization, methanol was added to the reaction mixture to precipitate the nanocapsules. Several drops of aqueous NaCl solution were added to methanol solution to increase aggregation of the nanocapsules. The nanocapsules were separated from the reaction mixture and purified in repeated centrifugation and resuspension steps using methanol, water-methanol mixture and water as washing solutions.

Nanocapsules were suspended in a mixture of 1 ml of methanol and 1 ml of aqueous 1M NaOH, and the mixture was stirred at 75 °C for 1 hour to hydrolyze pore-forming templates. Hydrolysis was monitored with LC-MS. After the hydrolysis, the mixture was neutralized with aqueous HCl and the nanocapsules were precipitated with methanol and washed with methanol.

Trimerization of Isocyanate in $CDCl_3$. 100 μl of p-tolyl isocyanate was dissolved in 1 ml of deuterated chloroform in a small vial. About 2.5 mg of proazaphosphatrane (Verkade's superbase) was weighed out in another small vial with a small stir bar. Isocyanate soultion in $CDCl_3$ was added to the catalyst vial. Since the reaction was taking place in $CDCl_3$, the solution (including trimer of isocynaurates and $CDCl_3$) was directly transferred into NMR sample tube and analysed. For LC/MS characterization, an aliquot was diluted in methanol and analysed. The same procedure was followed for other substrates.

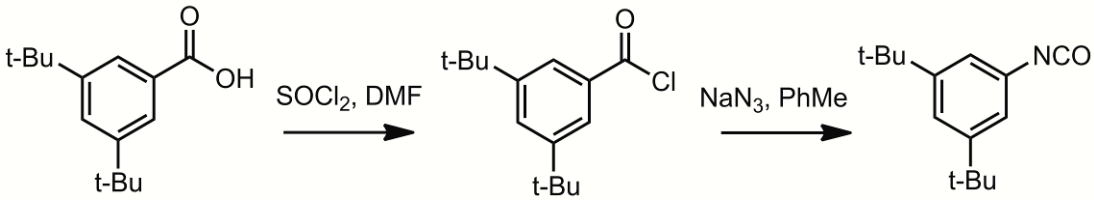
Trimerization of Isocyanate in C_6D_6. 100 μl of p-tolyl isocyanate was dissolved in 1 ml of deuterated benzene in a small vial. About 2.5 mg of proazaphosphatrane (Verkade's superbase) was weighed out in another small vial with a small stir bar.

Isocyanate soultion in C_6D_6 was added to the catalyst vial. A precipitate was formed after 3 min, and was washed 3 times with benzene. The precipitate was filtered and dried under vacuum for 1 hr. Crude product (isocynaurate) was dissolved in $CDCl_3$ and transferred into an NMR tube and analysed. For LC/MS characterization, an aliquot was diluted in methanol and analysed. The same procedure was followed for other substrates.

Trimerization of isocyanates inside nanocapsule. A suspension of nanocapsules prepared as described above (20 mg of dry residue), was mixed with 1 ml of $CDCl_3$ in a small vial. Isocyanate solution in $CDCl_3$ was added to this suspension. At regular intervals, the mixture was transferred to an NMR tube and an ^1H NMR spectrum was acquired. For LC/MS characterization, an aliquot was diluted in methanol and centrifuged for 10 min at 8000 RPM for sedimentation of nanocapsules, and the supernatant was analysed. The same procedure was followed for other substrates. Nanocapsules were washed 3 times in methanol and precipitated using a centrifuge, then were redispersed in $CDCl_3$, and the reaction with isocyanates was repeated.

Synthesis of 3,5-di-*tert*-butylyphenyl isocyanate. 4.43 mmol (1.039 g) of 3,5-di-*tert*-butyl benzoic acid was dissolved in thionyl chloride (3.0 ml) in a small round bottom flask. One drop of DMF was added to facilitate the reaction. The flask was equipped with a drying tube. The solution was stirred at ambient temperature for 45 minutes. The excess of thionyl chloride was evaporated on a rotary evaporator; the solid residue was then dissolved in toluene (~5.0 ml), and the solvent was evaporated to dryness. This step was repeated to ensure removal of volatile reaction products and unreacted thionyl chloride.

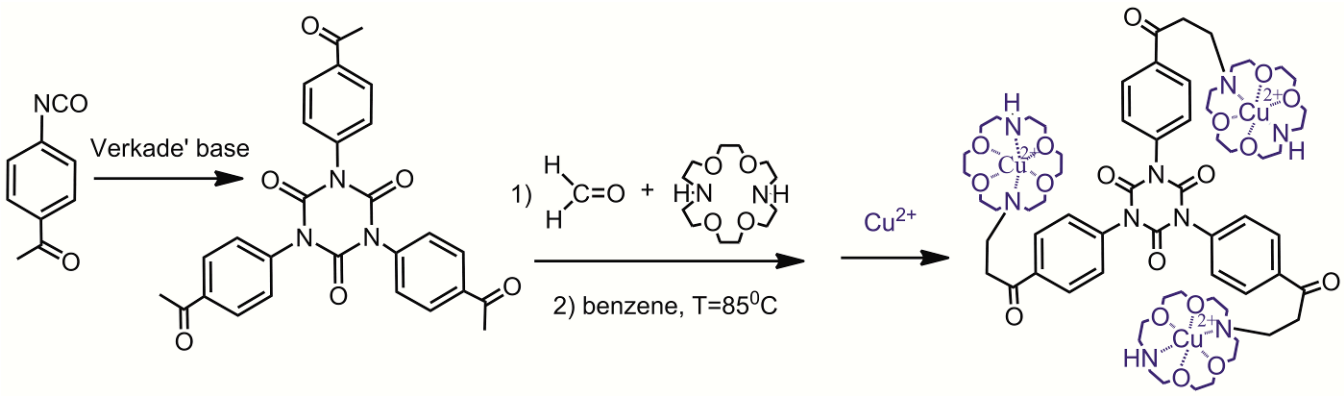
9.0 mmol of sodium azide (0.60 g, ~ 2 fold excess of 3,5-di-*tert*-butyl benzoic acid chloride) was dissolved in 15.0 ml toluene. The solution was added to the crude product from previous step, and the mixture was refluxed overnight. The condenser was also equipped with drying tube. The solvent was evaporated. The mixture was filtered by using use a gravity filtration using a Pasteur pipette with a cotton ball and was washed with hexane. Evaporation of the solvent under reduced pressure gave the crude product. No further purification was required.



Trimerization of 3,5-di-*tert*-butylyphenyl isocyanate. 50.0 µl of isocyanate was dissolved in 1.0 ml of deuterated chloroform in a small vial. In another small vial, ~3.5 mg of proazaphosphatrane (Verkade's base) was weighed out. Isocyanate solution was added to the catalyst vial. The solution mixture was stirred at room temperature. Progress of the reaction was monitored with NMR after 30, 60, 120, 240 min.

Synthesis of an entrapped trimer from 4-acetylphenyl isocyanate followed by a functionalization with diaza-18-crown-6. One portion of nanocapsules containing entrapped Varkade's superbase was dissolved in 1 ml of benzene. 10 mg of 4-acetylphenyl isocyanate was dissolved in 0.5 ml of benzene, and was added to nanocapsules solution. The solution mixture was stirred at room temperature for 6 hours. Nanocapsules were washed with methanol to remove unreacted chemicals. $4.32x10^{-2}$ mmol (11.34 mg) of diaza-18-crown-6 (DA18C6) was dissolved in 1ml of benzene, and added to the solution of nanocapsules. The mixture was stirred at ambient temperature for 12h to allow DA18C6 diffuse into the capsules. Then a solution of 0.13 mmol (4.0 mg) of paraformaldehyde in 1ml of benzene was added and was thoroughly mixed with nanocapsules solution. The reaction mixture was refluxed in an N_2 atmosphere for 12 h. The excess of solvent was evaporated under N_2 gas. Then nanocapsules were washed with methanol to remove unreacted chemicals.

Complexation of copper by entrapped crown compounds. 327 mg of $Cu(NO_3)_2$ was dissolved in a mixture of MeOH and C_6H_6 (1:2). 2 ml of metal solution was added to the 2 ml of nanocapsules solution. After one hour, nanocapsules were washed three times in methanol to remove non-complexed copper salt as judged by the clear supernatant.



Control experiments on diffusion of free metal salts and their complexes with DA18C6 into porous hollow nanocapsules. Nanocapsules (or nanocapsules with trimer inside) (30 mg) were dispersed in the solution of $Cu(NO_3)_2$ in methanol (2 mL, 0.5 mmol), and the suspension was kept at ambient conditions for 1 hour. After the incubation of nanocapsules with the solution of copper nitrate, the suspension was centrifuged at approx. 2,000 g for 5 minutes. Nanocapsules appear colored. The supernatant was withdrawn with a Pasteur pipette so as to leave the precipitated nanocapsules intact. Methanol (2 mL) was added slowly so as not to disturb the precipitated nanocapsules. The precipitate remains colored due to the presence of $Cu(NO_3)_2$ in the interior of nanocapsules. The precipitated nanocapsules were resuspended in methanol by agitation using a vortexer for 1 minute on a medium setting. The nanocapsules were precipitated using a centrifuge at approx. 2,000 g for 5 minutes, supernatant was removed, and the precipitate was resuspended and precipitated again as described above. The precipitate now appeared colorless suggesting that $Cu(NO_3)_2$ was washed away from nanocapsules. Samples containing entrapped complex, described in the main text, do not become discolored under similar processing. This experiment confirmed that the free metal salts diffuse in and out of nanocapsules and that they do not bind to nanocapsules or a trimer inside nanocapsules.

Similarly, nanocapsules (or nanocapsules with trimer inside) were dispersed in a solution of the complexes between $Cu(NO_3)_2$ and DA18C6 in methanol. The complexes were prepared by the standard literature procedures using the same concentration of metal salts as above, 0.5 mmol. The color of the solution corresponded to the complexed metal. The nanocapsules were incubated with the solution of these complexes for two hours. The incubation time was 2-4 times longer than the exposure of nanocapsules to free DA18C6 before the addition of metal salts or the exposure to free metal salts described above. After that, the nanocapsules were precipitated using a centrifuge at approx. 2,000 g for 5 min. The precipitate appeared white. The supernatant was withdrawn carefully with Pasteur pipette, and fresh methanol was added to the precipitate. In contrast with the experiment above, the precipitated nanocapsules showed no coloration. This experiment suggested that the complex between $Cu(NO_3)_2$ and DA18C6 did not enter the nanocapsules.

Similarly, nanocapsules with trimer inside were dispersed in a solution of DA18C6 in benzene and the suspension was kept at ambient conditions (or at 80°C) for 10 hours. Sample was precipitated with methanol for removing of unreacted compounds and was redispersed in the solution of $Cu(NO_3)_2$ in methanol/benzene (2 mL, 0.5 mmol), and the suspension was kept at ambient conditions for 1 hour. After the incubation of nanocapsules with the solution of copper nitrate, more methanol was added and the suspension was centrifuged at approx. 2,000 g for 5 minutes. Nanocapsules appear colored. The supernatant was withdrawn with a Pasteur pipette so as to leave the precipitated nanocapsules intact. Methanol (2 mL) was added slowly so as not to disturb the precipitated nanocapsules. The precipitate remains colored due to the presence of $Cu(NO_3)_2$ in the interior of nanocapsules. The precipitated nanocapsules were resuspended in methanol by agitation using a vortexer for 1 minute on a medium setting. The nanocapsules were precipitated using a centrifuge at approx. 2,000 g for 5 minutes, supernatant was removed, and the precipitate was resuspended and precipitated again as described above. The precipitate now appeared colorless suggesting that $Cu(NO_3)_2$ was washed away from nanocapsules. Samples containing entrapped complex, described in the main text, do not become discolored under similar processing. This experiment confirmed that the DA18C6 did not stay inside without reaction with trimer (by the Mannich reaction) and washed away with centrifugation, and free metal salts diffuse in and out of nanocapsules and that they do not bind to nanocapsules or trimer inside nanocapsules.

LC–MS analyses were done on a Shimadzu (Shimadzu, Japan) instrument. The HPLC analytical column was an Nova-Pak C18 reverse phase column (150 mm×3.9 mm i.d., 4 μm, Waters, Ireland) maintained at 25°C in the column oven. An isocratic flow was delivered via a binary pump, in a composition of 5% of eluent A (water/formic acid (100:0.1, v/v)) and 95% methanol (v/v), at flow rate of 0.2 ml/min.

^{1}H NMR spectra were recorded on a Bruker spectrometer at 400 MHz. The chemical shifts were recorded in δ relative to the residual solvent peaks at 7.27 (^{1}H for $CDCl_3$) and 7.16 (^{1}H for C_6D_6).

Dynamic Light Scattering. Hydrodynamic diameter and polydispersity index (PDI) measurements were performed on a Malvern Nano-ZS zetasizer (Malvern Instruments Ltd., Worcestershire, U.K.). The Helium-Neon laser, 4mW, operated at 633 nm, with the scatter angle fixed at 173º, and the temperature at 25 ºC. 80 μL samples were placed into disposable cuvettes without dilution (70 μL, 8.5 mm center height Brand UV-Cuvette micro). Each data point was an average of 10 scans.

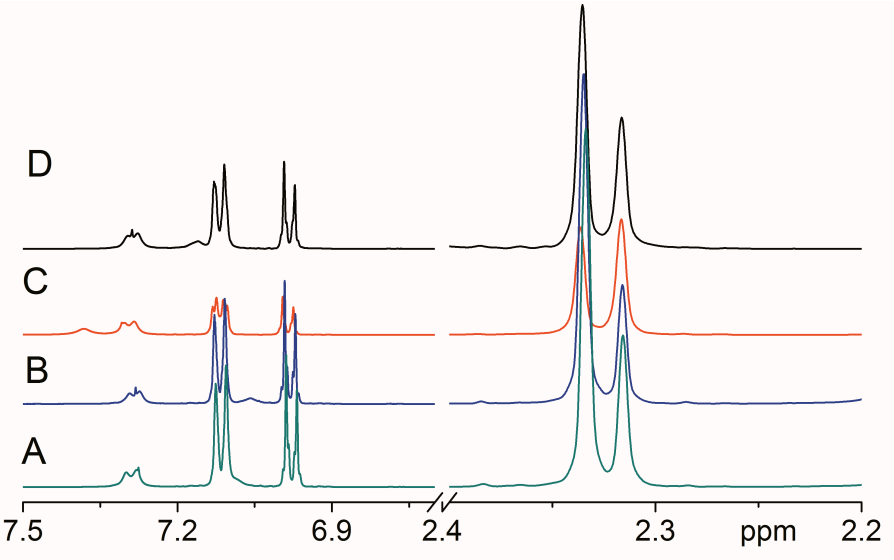


Figure 1S. Partial ^{1}H NMR spectra (400 MHz, $CDCl_3$, 298K) of a reaction mixture of p-tolyl isocyanate with nanocapsules with encapsulated catalyst after 1 hour of reaction first (A), second (B), third (C) and fourth (D) runs; peaks of methanol omitted for clarity.

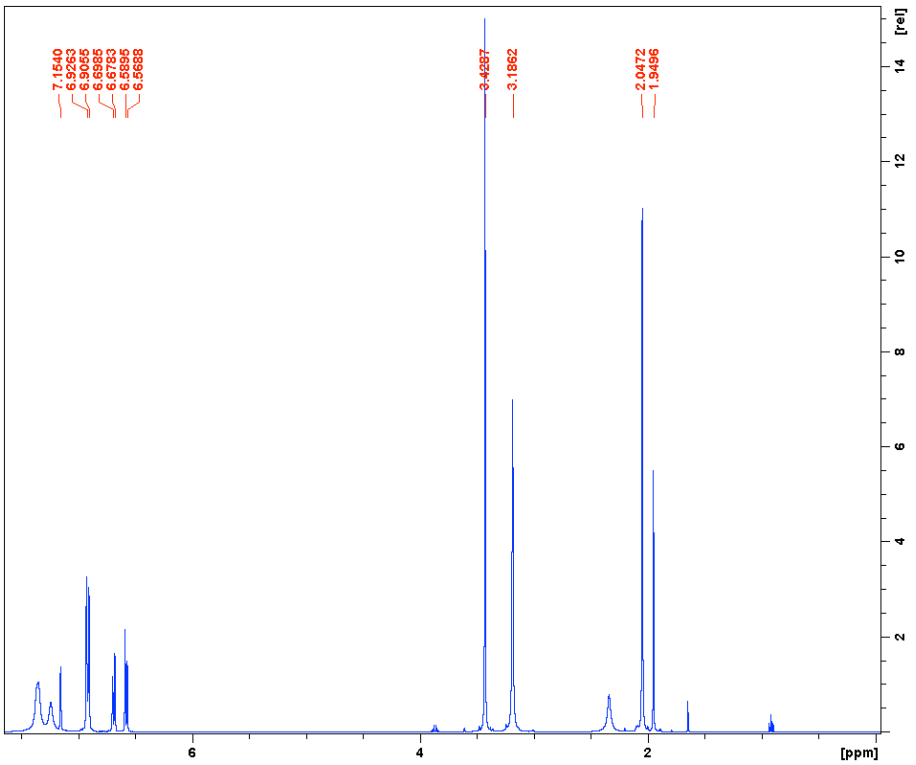


Figure 2S. Cyclotrimerization of p-tolyl isocyanate. ^{1}H NMR spectrum (400 MHz, C_6D_6, 298K) of reaction mixture of p-tolyl isocyanate with nanocapsules containing encapsulated catalyst after 30 min. of reaction.

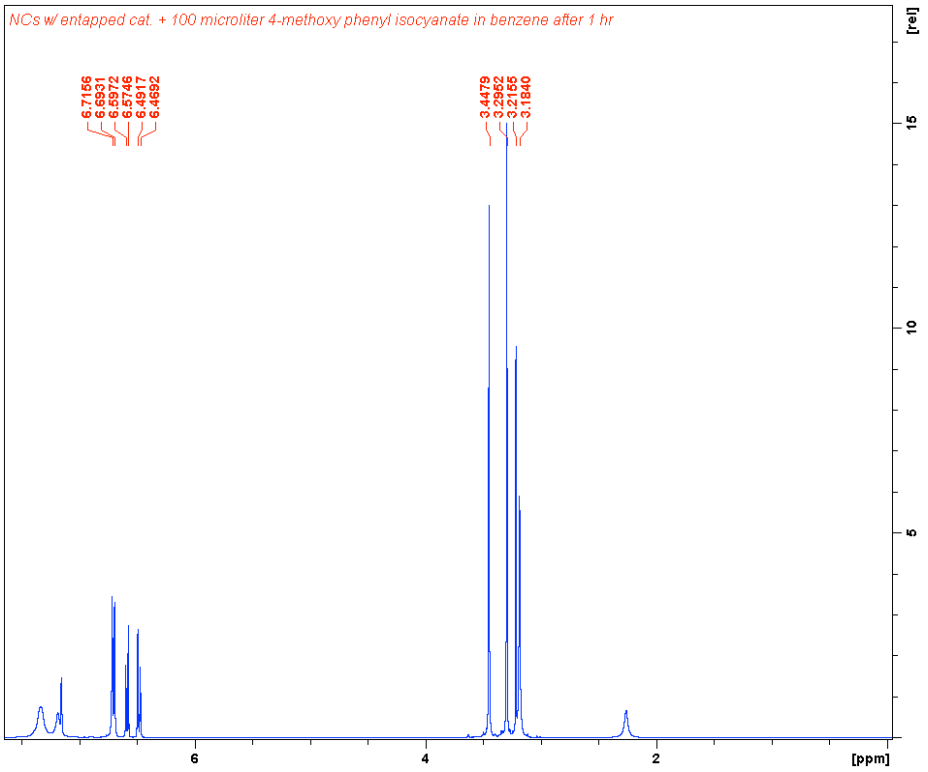


Figure 3S. Cyclotrimerization of 4-methoxyphenyl isocyanate. ^{1}H NMR spectrum (400 MHz, C_6D_6, 298K) of reaction mixture of 4-methoxyphenyl isocyanate with nanocapsules containing encapsulated catalyst after 30 min. of reaction.

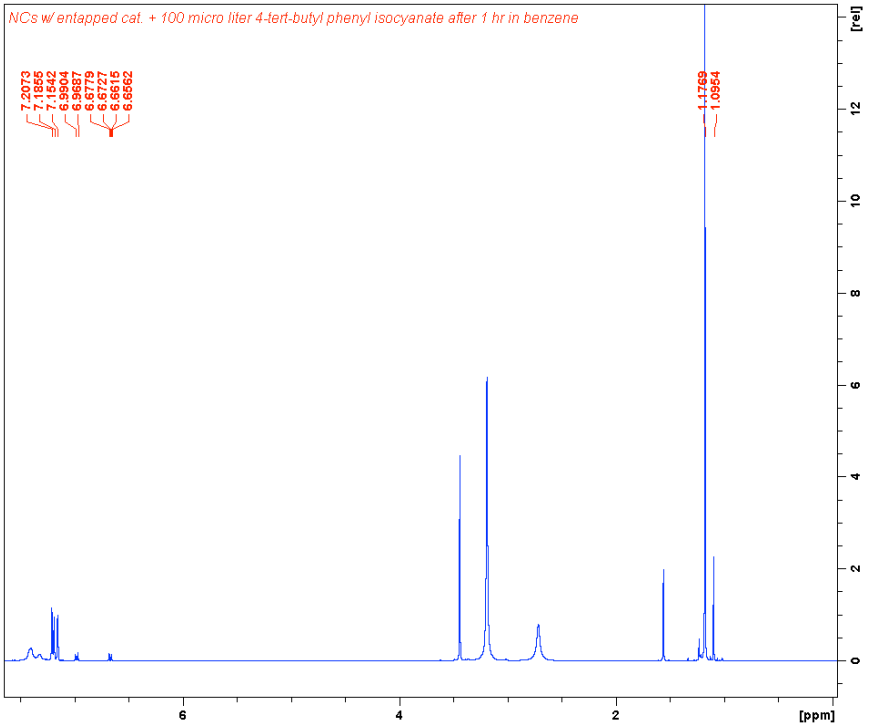


Figure 4S. Cyclotrimerization of 4-tert-butylphenyl isocyanate. ^{1}H NMR spectrum (400 MHz, C_6D_6, 298K) of reaction mixture of 4-tert-butylphenyl isocyanate with nanocapsules containing encapsulated catalyst after 30 min. of reaction.

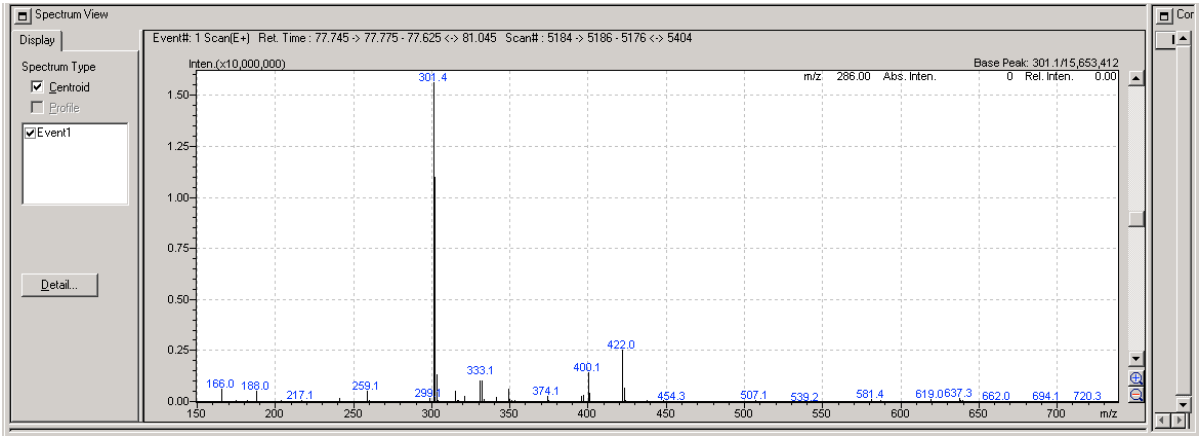


Figure 5S. LC-MS of solution after trimerization of p-tolyl isocyanate after 20 min

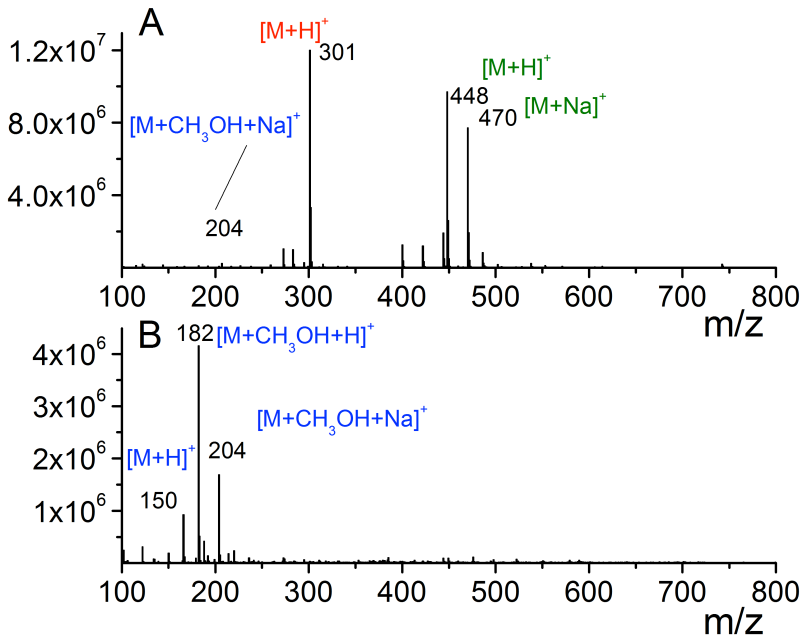


Figure 6S. (A) Mass spectrum of methanol solution of isocyanurate formation in the presence of proazaphosphatrane as catalyst. Trimer (green), catalyst (red), and starting material (blue) are detected. (B) Mass spectrum of methanol supernatant after precipitation of nanocapsules following the reaction of 4-methoxyphenyl isocyanate in the presence of nanocapsules containing entrapped proazaphosphatrane, corresponding to the NMR spectrum on Figure 3S. Only starting material (blue) is detected in the supernatant.

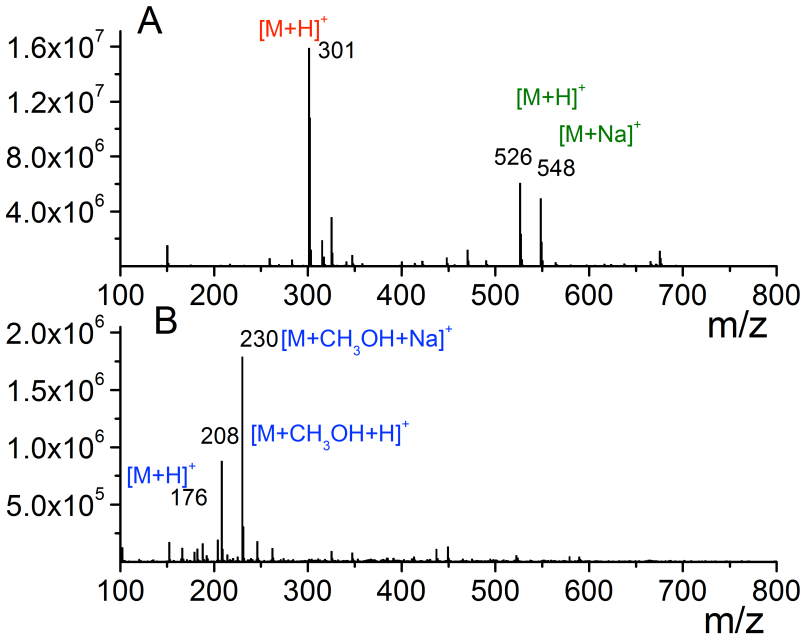


Figure 7S. (A) Mass spectrum of methanol solution of isocyanurate formation in the presence of proazaphosphatrane as catalyst. Trimer (green), catalyst (red), and starting material (blue) are detected. (B) Mass spectrum of methanol supernatant after precipitation of nanocapsules following the reaction of 4-tert-butylphenyl isocyanate in the presence of nanocapsules containing entrapped proazaphosphatrane, corresponding to the NMR spectrum on Figure 4S. Only starting material (blue) is detected in the supernatant.

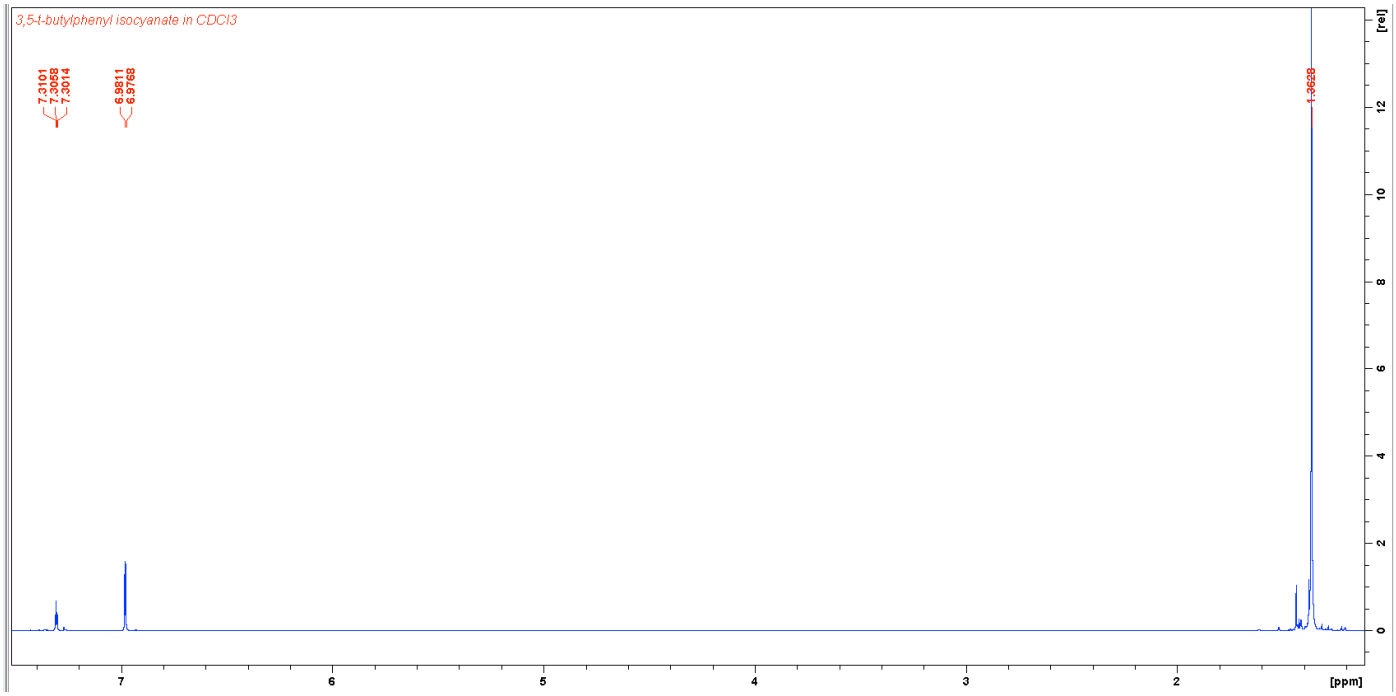


Figure 8S. ^{1}H NMR of 3,5-Di-tert-butylphenyl isocyanate

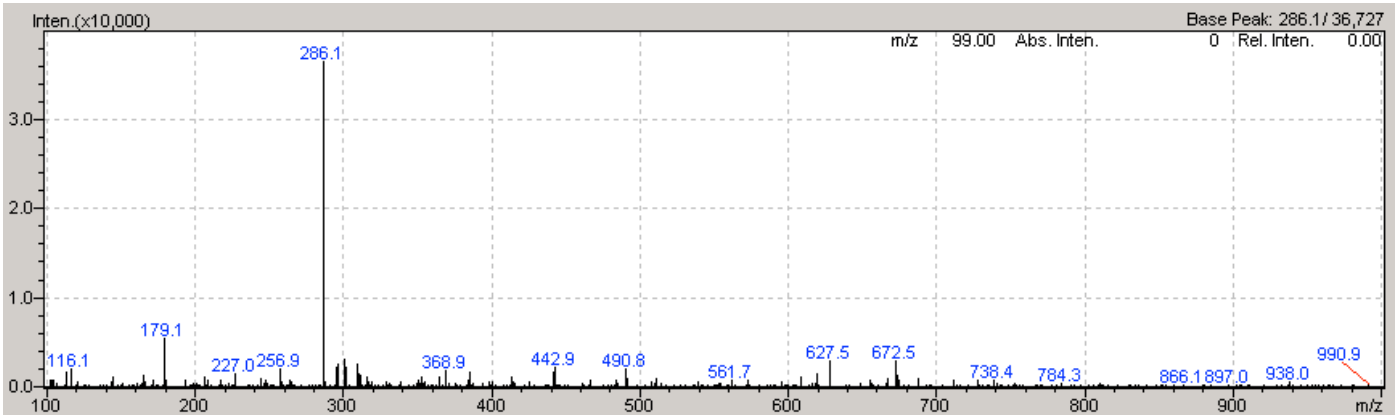


Figure 9S. LC-MS of 3,5-Di-tert-butylphenyl isocyanate

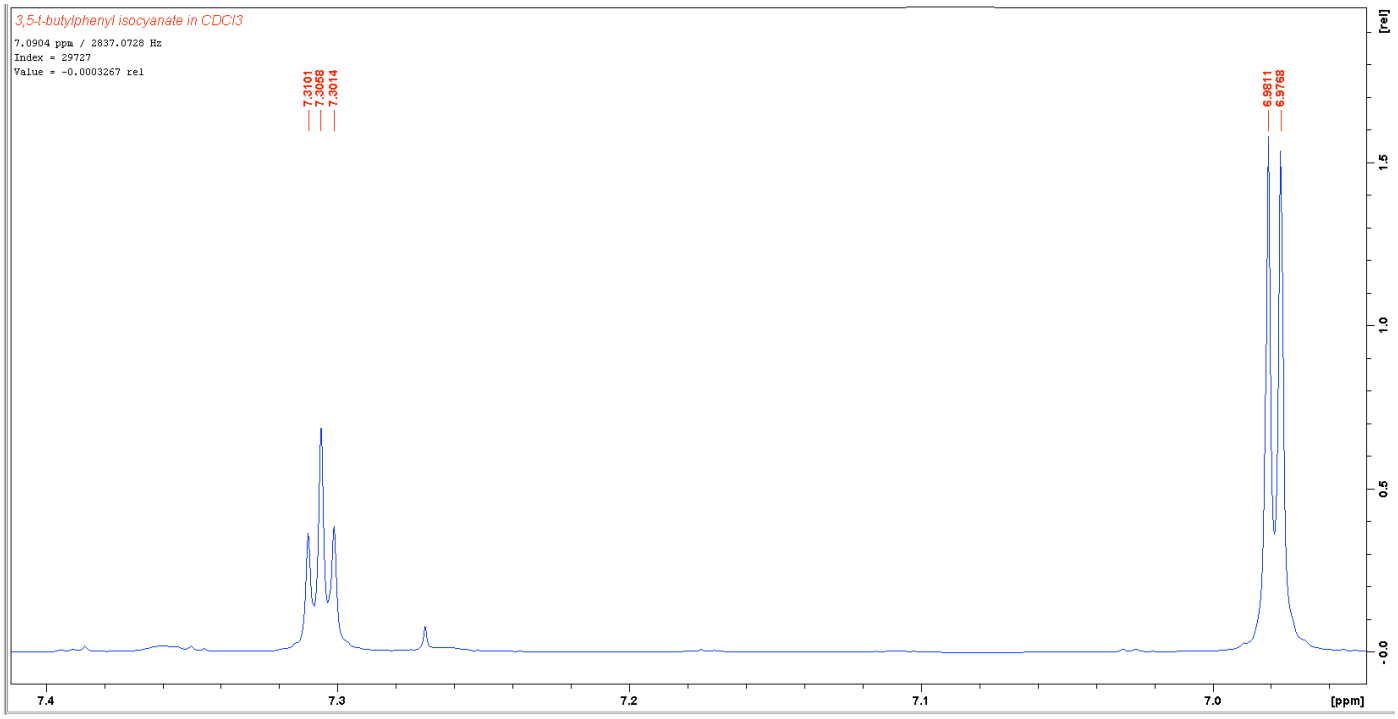


Figure 10S. ^{1}H NMR of trimerization of 3,5-Di-tert-butylphenyl isocyanate after 30 min

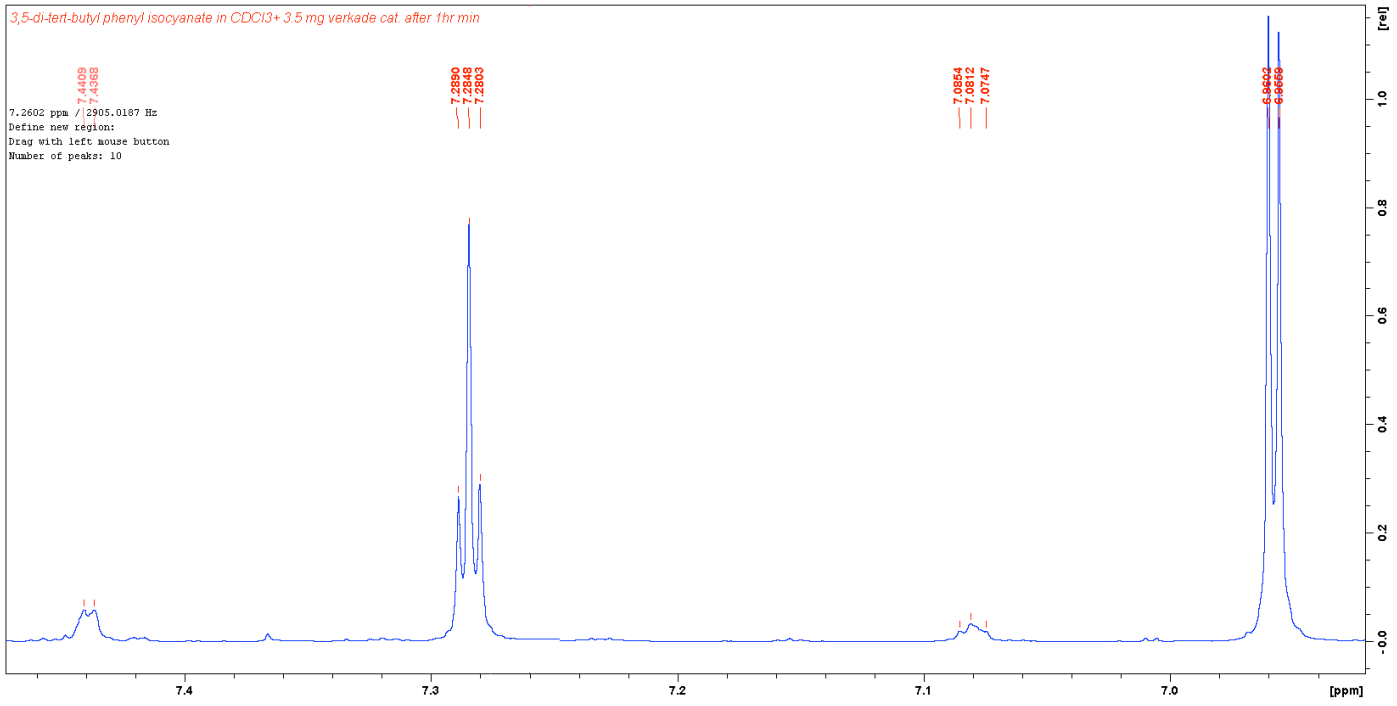


Figure 11S. ^{1}H NMR of trimerization of 3,5-Di-tert-butylphenyl isocyanate after 60 min

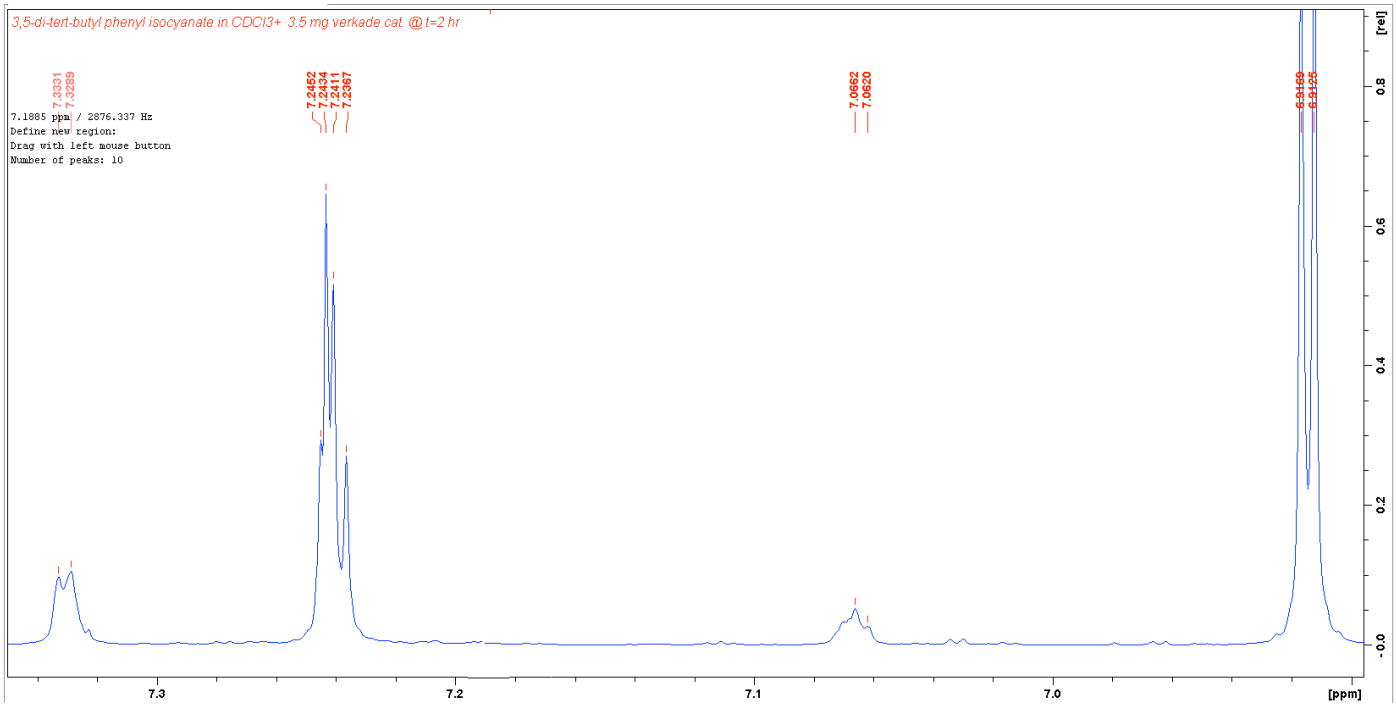


Figure 12S. ^{1}H NMR of trimerization of 3,5-Di-tert-butylphenyl isocyanate after 120 min

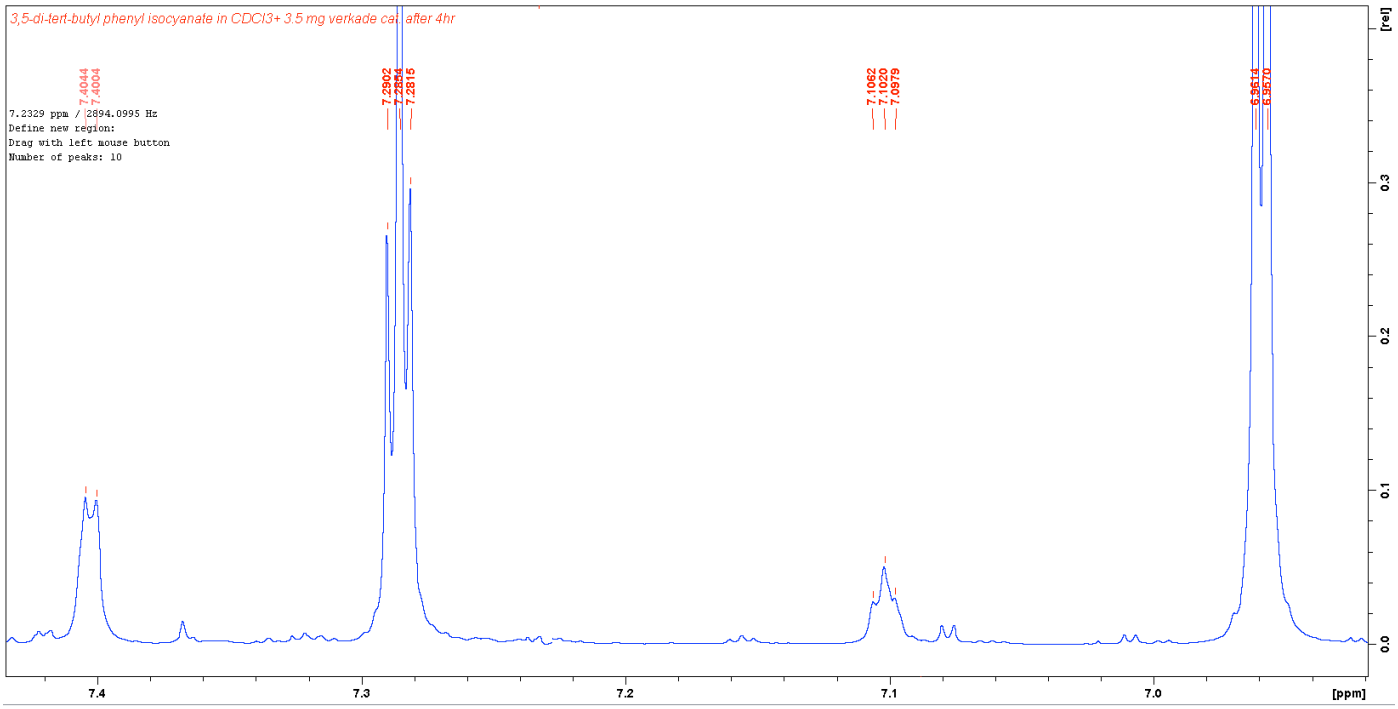


Figure 13S. ^{1}H NMR of trimerization of 3,5-Di-tert-butylphenyl isocyanate after 240 min

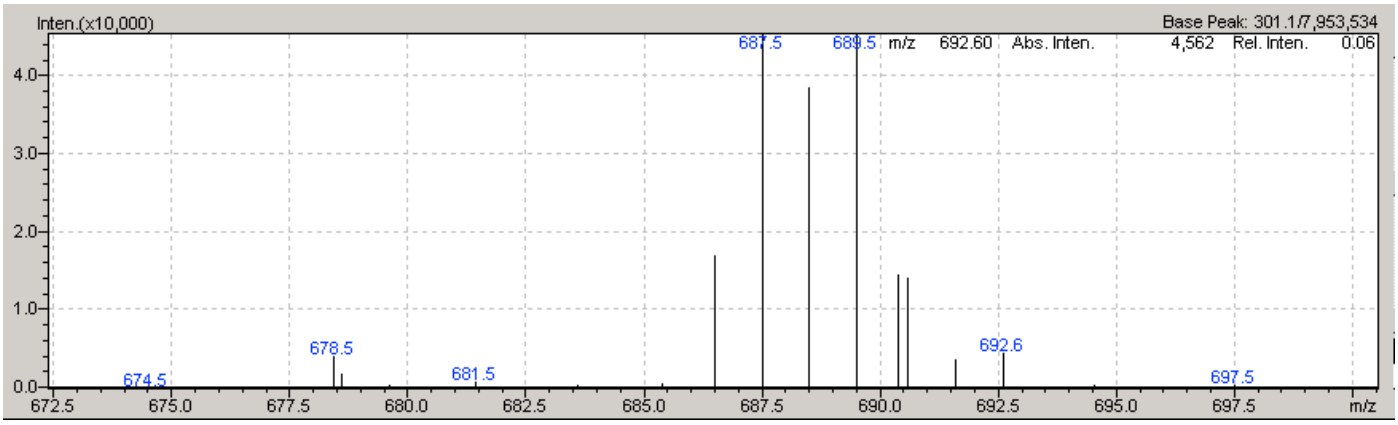


Figure 14S. LC-MS of solution after trimerization of 3,5-Di-tert-butylphenyl isocyanate after 3 h

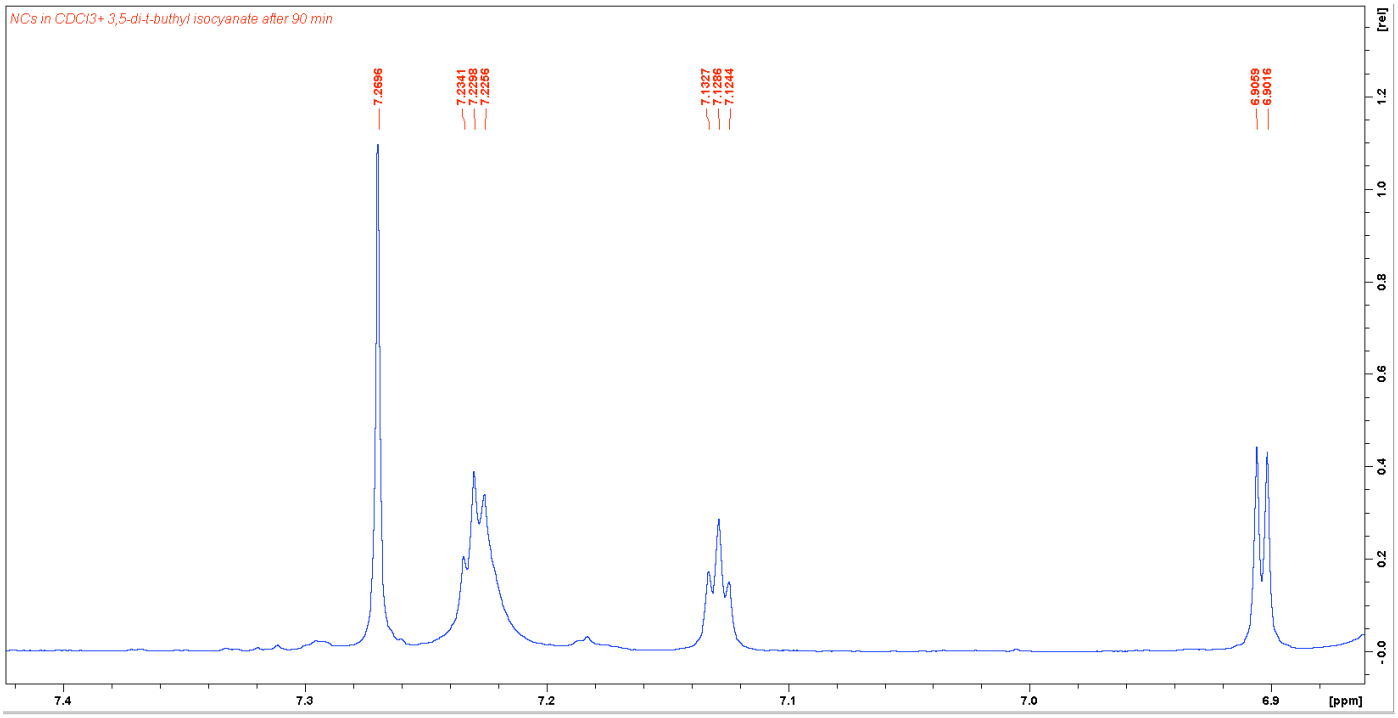


Figure 15S. ^{1}H NMR of trimerization of 3,5-Di-tert-butylphenyl isocyanate in the present of nanocapsules after 90 min

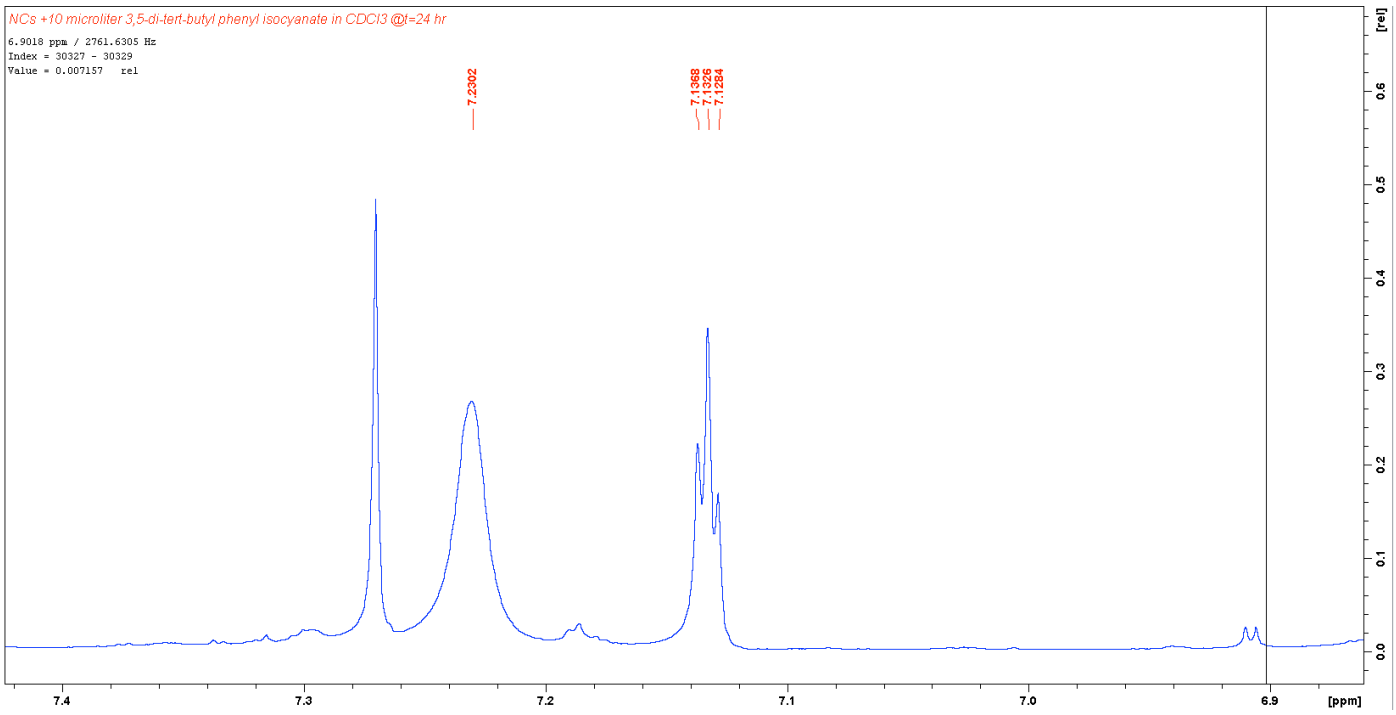


Figure 16S. ^{1}H NMR of trimerization of 3,5-Di-tert-butylphenyl isocyanate in the present of nanocapsules after 24h

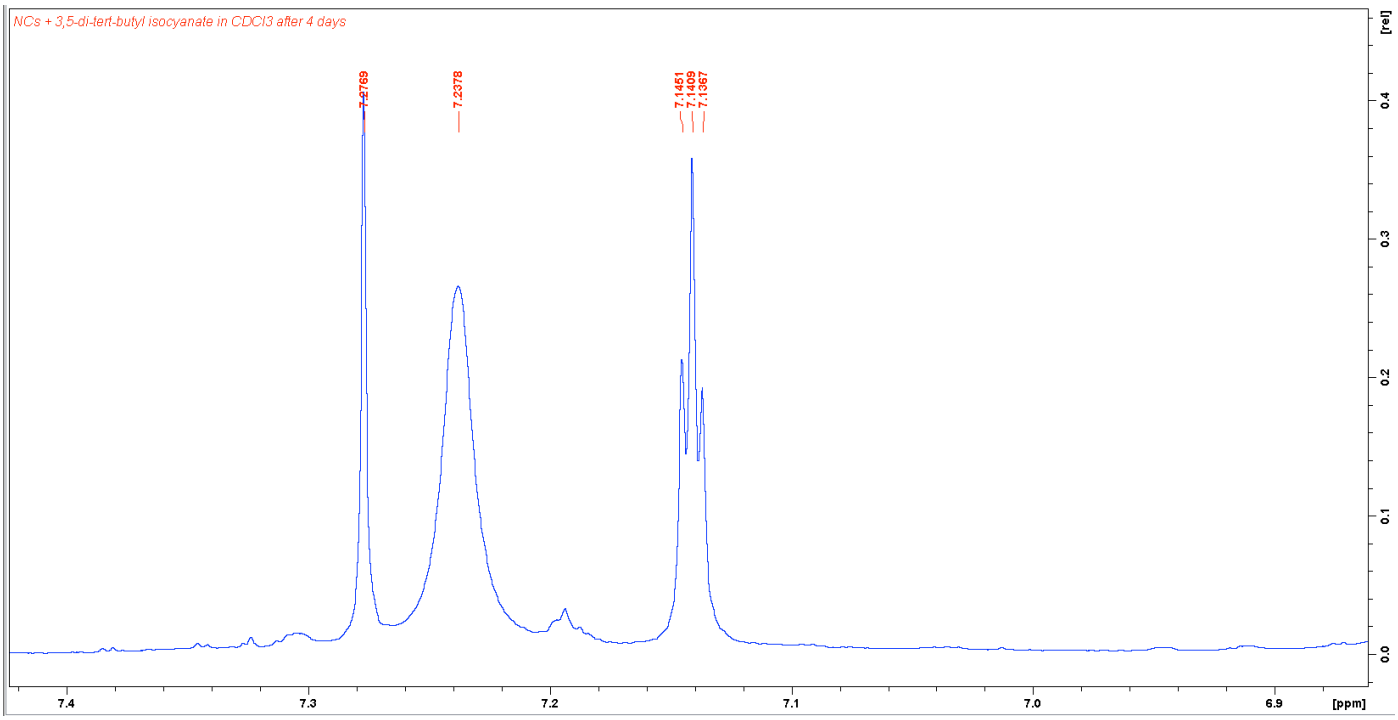


Figure 17S. ^{1}H NMR of trimerization of 3,5-Di-tert-butylphenyl isocyanate in the present of nanocapsules after 4 days

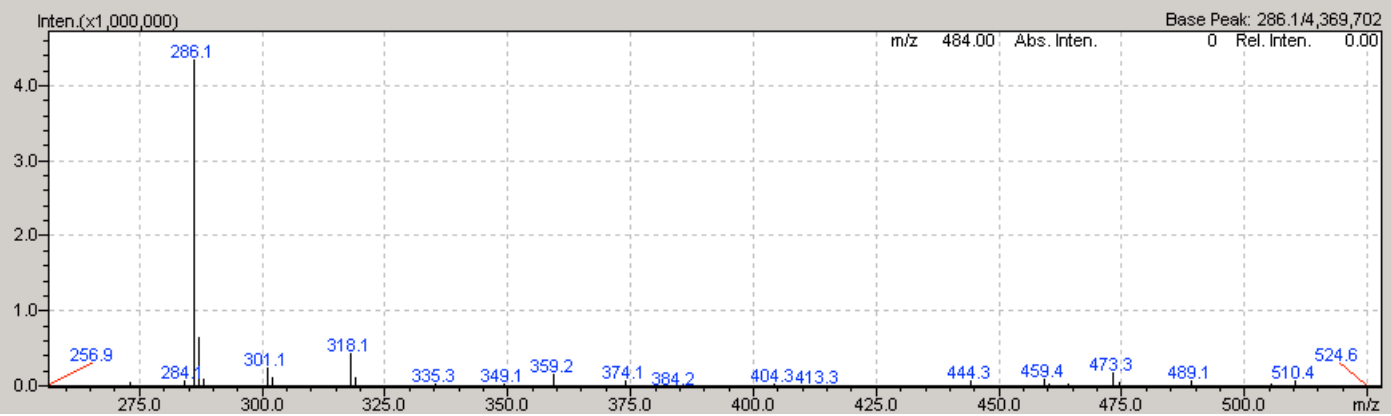


Figure 18S. LC-MS of supernatant after trimerization of 3,5-Di-tert-butylphenyl isocyanate in the present of nanocapsules after 4 days