Magnetic Iron Oxide Nanoparticle Functionalization: the Isocyanate Moiety as a Suitable Monodentate Anchoring Group

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Supporting Information

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I. Materials and methods

Unless otherwise noted, all reactions were performed under dry nitrogen in oven-dried glassware with standard vacuum line techniques. Isocyanates **7-11** and **16** were purchased from Sigma Aldrich and used without further putification. *p*-Maleimidophenyl isocyanate (PMPI) **12** was prepared according to the previously reported procedure.[i]

Dry toluene over molecular sieves and other solvents (P.A., ACS reagent) were purchased from Sigma Aldrich.

γ-Fe_2O_3 SuperParamagnetic Iron Oxide Nanoparticles (SPIONs) with an average diameter of 10 ± 2 nm were purchased from Alpha Aesar and stored away from moisture.

Dry nanoparticles were obtained using a vacuum drying chamber Büchi GKR-S1 and stored under dry atmosphere.

A sonicator Elma Elmasonic S 30H (ultrasonic frequency 37 kHz, ultrasonic power effective 80 W, ultrasonic peak performance maximum 320 W with standard sine-wave modulation) was used to perform reactions with nanoparticles.

Elemental Analysis were performed on a Perkin Elmer 2400 Series II analyzer in CHN mode.

FTIR spectra of nanoconjugates **1-6**, **13-15** and **17** were acquired on a Spectrum One FTIR spectrophotometer (Perkin Elmer) in the range 4000-450 cm^{-1} in transmittance mode. 0.5 mg of sample were diluted in 150 mg FTIR grade KBr and ground in an agate mortar. KBr was previously dried at 125 °C under vacuum. Then mixed powder was pressed at 9 tons for 3 min to obtain slim semitransparent tablet. Spectra were recorded selecting 64 scans and 4 cm^{-1} resolution. The background of pure KBr was separately recorded using the same conditions and automatically subtracted from sample spectra. Spectra of starting isocyanate **7-12** and **16** were acquired in transmittance mode in the range 4000-600 cm^{-1} using NaCl windows as support and selecting 4 scans and 4 cm^{-1} resolution.

The NMR spectrometer with the HR-MAS facility, located in the "Centro Interdipartimentale Grandi Apparecchiature" (C.I.G.A.), of University of Milan, is an FT-NMR *Avance*^{TM} *500* (*Bruker Italia S.r.l.*) with a superconducting ultrashield magnet of 11.7 Tesla (^{1}H frequency: 500.13 MHz).

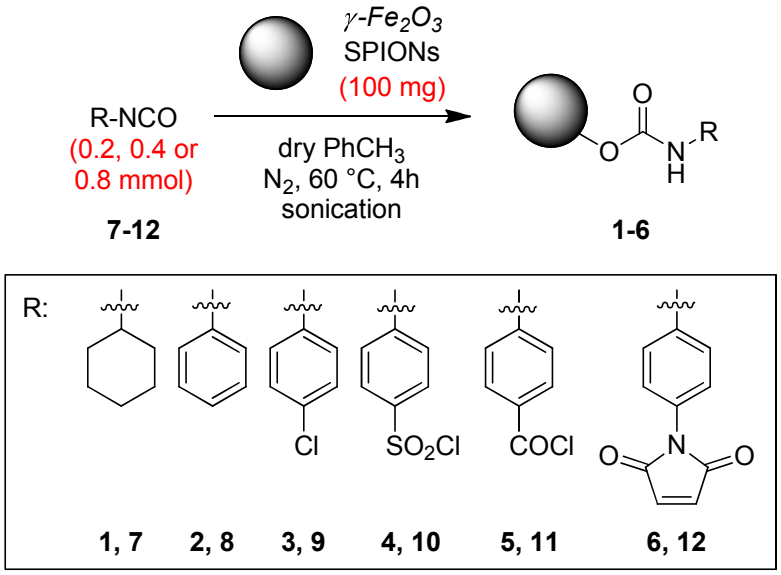
The probe is doubly tuned (^{1}H and ^{13}C), in addition to a 2H lock channel. All three channels are operating via a single NMR transmit/receive solenoid coil located inside the MAS turbine. The probe is capable of performing either direct or indirect (inverse) detection experiments. The probe allows to perform high resolution MAS experiments at spinning rates of up to 15 KHz, with 4 mm zirconia oxide rotors, for liquid or liquid-like samples; in our study the spinning rates were optimized between 5 KHz and 12 KHz speed values to rich a compromise between the needing to have the spinning side bands out of the ^{1}H spectrum, the best resolution behaviour and the minimum presence of turbulence or rotational artifacts.

All the samples were diluted in deuterated methyl sulfoxide (few milligrams in 150-200 µl DMSO-d_6 100%); the saturated solutions (do to the low solubility of γ-Fe_2O_3 nanoconjugates) of nanoparticles dispersion, after different cycles of vortex/sonication, were decanted by night leaving to precipitate the large aggregates and the upper suspension was then collected for NMR analysis. A dilution at different levels was sometimes necessary to find the concentration limit before the broadening of the NMR signals. The sample temperature it's speed rotation dependent and was so varying from 30 to 35 °C, in different experiments performed.

II. Synthesis of nanoconjugates 1-6, 13-15 and 17

1. Synthesis of nanoconjugates 1-6

Nanoconjugates **1-6** were synthesized accordingly to the general procedure reported in *Scheme S1*:



Scheme S1

Naked SPIONs (100 mg) were suspended in dry toluene (5 ml) and sonicated for 30 min at 60 °C. To this suspension was then added the proper isocyanate **7-12** (0.2, 0.4 or 0.8 mmol) and the mixture was sonicated for 4 h at 60 °C. After this time, the suspension was centrifugated at 5000 r/min for 10 min and the supernatant was removed. The solid residue was washed with fresh $PhCH_3$ (3x5 ml), then with Et_2O (3x5 ml) and vortex/sonication/centrifugation were cyclic repeated for each washing. Functionalized NPs were dried on air overnight and then under vacuum for 24 h, to afford the corresponding nanoconjugates **1-6** as brown powders.

All nanoconjugates were characterized by E.A. (in order to determine the loading of the organic molecule onto NP) and FTIR spectroscopy, while only **1-3** and **6** have been also characterized by ^{1}H and ^{13}C HR-MAS NMR.

- ✓ *SPIONs*-Cyclohexyl Carbamate **1**

  Elemental Analysis E.A. in CHN mode: for organic residue $C_7H_{12}NO$, found: C 8.91, H 1.62, N 1.54, O (calculated on molar nitrogen percentage) 1.76% [0.2 mmol of **7**/100mg$_{NPs}$]; found: C 17.31, H 2.87, N 3.04, O (calculated on molar nitrogen percentage) 3.47% [0.4 mmol of **7**/100mg$_{NPs}$]; found: C 33.43, H 5.28, N 5.96, O (calculated on molar nitrogen percentage) 6.80% [0.8 mmol of **7**/100mg$_{NPs}$].

  Loadings from E.A.: 13.83%, corresponding to 1.10 mmol/g$_{NPs}$ [0.2 mmol of **7**/100mg$_{NPs}$]; 26.69%, corresponding to 2.11 mmol/g$_{NPs}$ [0.4 mmol of **7**/100mg$_{NPs}$]; 51.47%, corresponding to 4.08 mmol/g$_{NPs}$ [0.8 mmol of **7**/100mg$_{NPs}$].

  ^{1}H HR-MAS NMR (500 MHz , [D$_6$]DMSO, 4 KHz): δ= 5.57 (d, *J* = 5 Hz, 1H, NH carbamate), 3.33 (signal covered by water, assigned by 2D experiments, 1H, CH-N), 1.74-0.93 ppm (m, 10H, CH_2).

  ^{13}C all decoupled HR-MAS NMR (125 MHz, [D$_6$]DMSO, 4 KHz,): δ= 157.08 (C=O carbamate), 47.97 (CH), 36.71 (CH_2), 33.81 (CH_2), 25.79 (CH_2), 25.10 (CH_2), 24.92 ppm (CH_2).

  FTIR in trasmittance (blank KBr): 3326 (υ, N-H), 2928, 2850 (υ, C-H), 1626 (υ, C=O carbamate), 1573 (δ, N-H), 1311, 1243 (υ, C-O and C-N), 1088 (δ, C-H), 640-562 cm^{-1} (υ, Fe-O).

✓ *SPIONs*-Phenyl Carbamate **2**

Elemental Analysis E.A. in CHN mode: for organic residue C_7H_6NO, found: C 10.43, H 1.12, N 1.79, O (calculated on molar nitrogen percentage) 2.02% [0.2 mmol of **8**/100mg_{NPs}]; found: C 18.08, H 1.66, N 3.18, O (calculated on molar nitrogen percentage) 3.63% [0.4 mmol of **8**/100mg_{NPs}]; found: C 29.77, H 2.48, N 5.39, O (calculated on molar nitrogen percentage) 6.16% [0.8 mmol of **8**/100mg_{NPs}].

Loadings from E.A.: 15.36%, corresponding to 1.28 mmol/g_{NPs} [0.2 mmol of **8**/100mg_{NPs}]; 26.55%, corresponding to 2.21 mmol/g_{NPs} [0.4 mmol of **8**/100mg_{NPs}]; 43.80%, corresponding to 3.65 mmol/g_{NPs} [0.8 mmol of **8**/100mg_{NPs}].

^{1}H HR-MAS NMR (500 MHz, [D$_6$]DMSO, 6 KHz): δ= 8.64 (s, 1H, NH carbamate), 7.46 (d, *J* = 10 Hz, 2H, ArCH ortho to carbamate), 7.29 (m, J_A = 5, J_B = 10, 2H, ArCH meta to carbamate), 6.97 ppm (m, J_A = 5, J_B = 10, 1H, ArCH para to carbamate).

^{13}C all decoupled HR-MAS NMR (125 MHz, [D$_6$]DMSO, 6 KHz): δ= 153.01 (C_q, C=O carbamate), 140.17 (C_q, ArC-N), 129.25 (CH, ArCH ortho to carbamate), 122.28 (CH, ArCH para to carbamate), 118.67 ppm (CH, ArCH meta to carbamate).

FTIR in trasmittance (blank KBr): 3326 (υ, N-H), 3282, 3192 (υ, ArC-H), 1647 (υ, C=O carbamate), 1594, 1551 (δ, N-H), 1497, 1447, 1439 (υ, C=C), 1314, 1231 (υ, C-O and C-N), 753, 696 (δ, C-H out of plane), 640-560 cm^{-1} (υ, Fe-O).

✓ *SPIONs*-(*p*-Chloro)phenyl Carbamate **3**

Elemental Analysis E.A. in CHN mode: for organic residue C_7H_5NOCl, found: C 8.61, H 0.86, N 1.53, O (calculated on molar nitrogen percentage) 1.75, Cl (calculated on molar nitrogen percentage) 3.87% [0.2 mmol of **9**/100mg_{NPs}]; found: C 15.63, H 1.31, N 2.76, O (calculated on

molar nitrogen percentage) 3.15, Cl (calculated on molar nitrogen percentage) 6.98% [0.4 mmol of **9**/100mg$_{NPs}$]; found: C 20.96, H 1.61, N 3.80, O (calculated on molar nitrogen percentage) 4.34, Cl (calculated on molar nitrogen percentage) 9.62% [0.8 mmol of **9**/100mg$_{NPs}$].

Loadings from E.A.: 16.62%, corresponding to 1.08 mmol/g$_{NPs}$ [0.2 mmol of **9**/100mg$_{NPs}$]; 29.83%, corresponding to 1.93 mmol/g$_{NPs}$ [0.4 mmol of **9**/100mg$_{NPs}$]; 40.33%, corresponding to 2.61 mmol/g$_{NPs}$ [0.8 mmol of **9**/100mg$_{NPs}$].

^{1}H HR-MAS NMR (500 MHz, [D$_6$]DMSO, 6 KHz): δ= 8.83 (s, 1H, NH carbamate), 7.48 (d, *J* = 10, 2H, ArCH meta to carbamate), 7.33 ppm (d, *J* = 10, 2H, ArCH ortho to carbamate).

^{13}C all decoupled HR-MAS NMR (125 MHz, [D$_6$]DMSO, 6 KHz): δ= 152.81 (C$_q$, C=O carbamate), 139.01 (C$_q$, ArC-N), 129.09 (CH, ArCH meta to carbamate), 125.98 (C$_q$, ArC-Cl), 120.32 ppm (CH, ArCH ortho to carbamate).

FTIR in trasmittance (blank KBr): 3294 (υ, N-H), 1632 (υ, C=O carbamate), 1590, 1559 (δ, N-H), 1491, 1439 (υ, C=C), 1395, 1298, 1236 (υ, C-O and C-N), 1085, 1012 (υ, ArCH-Cl), 823 (δ, C-H out of plane), 637-561 cm^{-1} (υ, Fe-O).

✓ *SPIONs*-(*p*-Chlorosulfonyl)phenyl Carbamate **4**

Beilstein test: positive (bright green flame).

Elemental Analysis E.A. in CHN mode: for organic residue $C_7H_5NO_3ClS$, found: C 6.61, H 0.80, N 1.09, O (calculated on molar nitrogen percentage) 3.73, Cl (calculated on molar nitrogen percentage) 2.76, S (calculated on molar nitrogen percentage) 2.49% [0.2 mmol of **10**/100mg$_{NPs}$]; found: C 7.35, H 1.03, N 1.23, O (calculated on molar nitrogen percentage) 4.21, Cl (calculated on molar nitrogen percentage) 3.11, S (calculated on molar nitrogen percentage) 2.81% [0.4 mmol of **10**/100mg$_{NPs}$]; found: C 8.29, H 1.07, N 1.34, O (calculated on molar

nitrogen percentage) 4.59, Cl (calculated on molar nitrogen percentage) 3.39, S (calculated on molar nitrogen percentage) 3.07% [0.8 mmol of **10**/100mg$_{NPs}$].

Loadings from E.A.: 17.54%, corresponding to 0.80 mmol/g$_{NPs}$ [0.2 mmol of **10**/100mg$_{NPs}$]; 19.74%, corresponding to 0.90 mmol/g$_{NPs}$ [0.4 mmol of **10**/100mg$_{NPs}$]; 21.75%, corresponding to 0.99 mmol/g$_{NPs}$ [0.8 mmol of **10**/100mg$_{NPs}$].

FTIR in trasmittance (blank KBr): 3294 (υ, N-H), 1699 (υ, C=O carbamate), 1589, 1533 (δ, N-H), 1495, 1402 (υ, C=C), 1328 (asym υ, SO_2), 1156 (sym υ, SO_2), 633-556 cm^{-1} (υ, Fe-O).

✓ *SPIONs*-(*p*-Chloroacyl)phenyl Carbamate **5**

Beilstein test: positive (bright green flame).

Elemental Analysis E.A. in CHN mode: for organic residue $C_8H_5NO_2Cl$, found: C 11.30, H 1.10, N 1.68, O (calculated on molar nitrogen percentage) 3.84, Cl (calculated on molar nitrogen percentage) 4.25% [0.2 mmol of **11**/100mg$_{NPs}$]; found: C 13.71, H 1.29, N 1.94, O (calculated on molar nitrogen percentage) 4.43, Cl (calculated on molar nitrogen percentage) 4.91% [0.4 mmol of **11**/100mg$_{NPs}$]; found: C 17.75, H 1.68, N 2.68, O (calculated on molar nitrogen percentage) 6.12, Cl (calculated on molar nitrogen percentage) 6.78% [0.8 mmol of **10**/100mg$_{NPs}$].

Loadings from E.A.: 22.17%, corresponding to 1.21 mmol/g$_{NPs}$ [0.2 mmol of **11**/100mg$_{NPs}$]; 25.74%, corresponding to 1.41 mmol/g$_{NPs}$ [0.4 mmol of **11**/100mg$_{NPs}$]; 35.01%, corresponding to 1.92 mmol/g$_{NPs}$ [0.8 mmol of **11**/100mg$_{NPs}$].

FTIR in trasmittance (blank KBr): 3297 (υ, N-H), 1772 (υ, C=O acyl chloride), 1681 (υ, C=O carbamate), 1593, 1531, 1512 (δ, N-H), 1410 (υ, C=C), 1316, 1237 (υ, C-O and C-N), 1176 (δ, C-H), 634-579 cm^{-1} (υ, Fe-O).

✓ *SPIONs*-(*p*-Maleimido)phenyl Carbamate **6**

Elemental Analysis E.A. in CHN mode: for organic residue $C_{11}H_7N_2O_3$, found: C 8.41, H 0.88, N 1.89, O (calculated on molar nitrogen percentage) 3.24% [0.2 mmol of **12**/100mg_{NPs}]; found: C 17.99, H 1.41, N 4.30, O (calculated on molar nitrogen percentage) 7.37% [0.4 mmol of **12**/100mg_{NPs}]; found: C 30.12, H 1.75, N 6.07, O (calculated on molar nitrogen percentage) 13.87% [0.8 mmol of **12**/100mg_{NPs}].

Loadings from E.A.: 14.42%, corresponding to 0.67 mmol/g_{NPs} [0.2 mmol of **12**/100mg_{NPs}]; 31.07%, corresponding to 1.44 mmol/g_{NPs} [0.4 mmol of **12**/100mg_{NPs}]; 51.81%, corresponding to 2.41 mmol/g_{NPs} [0.8 mmol of **12**/100mg_{NPs}].

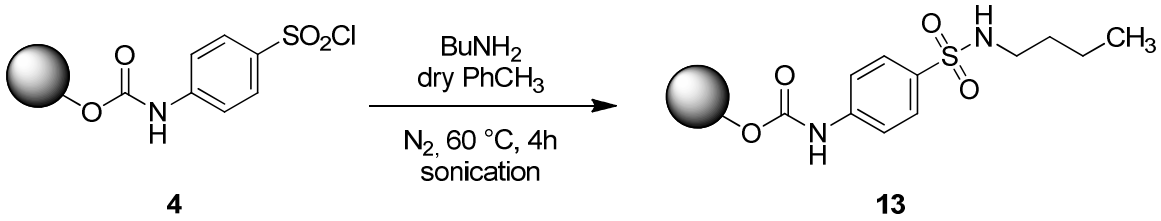
^{1}H HR-MAS NMR (500MHz, [D$_6$]DMSO, 12 KHz): δ= 8.94 (s, 1H, NH carbamate), 7.63 (d, *J* = 10, 2H, ArCH ortho to carbamate), 7.31 (d, *J* = 10, 2H, ArCH meta to carbamate), 7.21 ppm (s, 2H, maleimide vinyl).

^{13}C all decoupled HR-MAS NMR (125 MHz, [D$_6$]DMSO, 14 KHz): δ= 169.85 (C_q, C=O imide), 153.83 (C_q,C=O carbamate), 139.00 (C_q, ArC-NH carbamate), 134.39 (CH, maleimide), 127.19 (CH, ArCH ortho to carbamate), 120.01 (C_q, ArC-N maleimide), 118.36 ppm (CH, ArCH meta to carbamate).

FTIR in trasmittance (blank KBr): 3383 (υ, N-H), 3096 (υ, ArC-H), 1707 (υ, C=O imide), 1692 (υ, C=O carbamate), 1605, 1552 (δ, N-H), 1513 (υ, C=C), 1398 (υ, Ar-N), 1313 (υ, C-N imide), 1239-1175 (υ, C-O and C-N), 831 (δ, C-H), 689-559 cm^{-1} (υ, Fe-O).

2. Synthesis of nanoconjugates 13-15 and 17

✓ *SPIONs*-[*p*-(*N*-butylsulfamoyl)phenyl] Carbamate **13**



4 **13**

Scheme S2

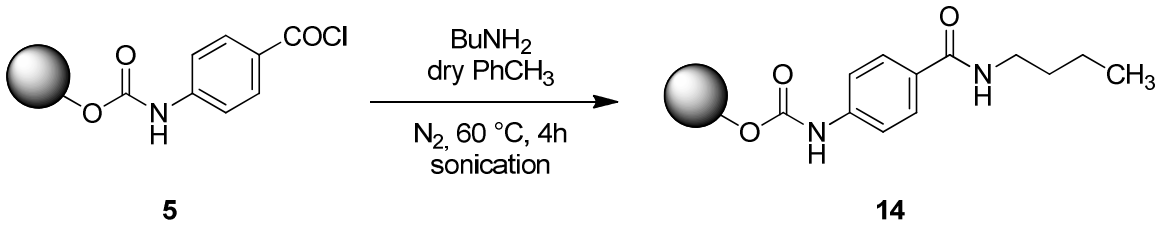
n-Butylamine (45 µl, 0.45 mmol, 5 eq) was added to a suspension of functionalized nanoparticles **4** (100 mg, loading 19.74% corresponding to 0.90 mmol/g_{NPs}, 1 eq) in dry toluene (5 ml), previously sonicated at 60 °C for 30 min; the mixture was then stirred, under sonication, at 60 °C for 4 h. After this time the suspension was centrifugated at 5000 r/min for 10 min and the supernatant was removed. The solid residue was washed with fresh $PhCH_3$ (3x5 ml), then with Et_2O (3x5 ml) and vortex/sonication/centrifugation were cyclic repeated for each washing. Functionalized NPs were dried on air overnight and then under vacuum for 24 h, to afford the nanoconjugates **13** as brown powders.

Elemental Analysis E.A. in CHN mode: for organic residue $C_{11}H_{15}N_2O_3S$, found: C 12.89, H 2.21, N 2.92, O (calculated on molar nitrogen percentage) 5.00, S (calculated on molar nitrogen percentage) 3.34%.

Loadings from E.A.: 26.36%, corresponding to 1.03 mmol/g_{NPs}.

FTIR in trasmittance (blank KBr): 2961-2874 (υ, aliphatic C-H), 1699 (υ, C=O carbamate), 1589, 1532 (υ, C=C), 1493, 1396 (δ, SO_2N-H), 1311 (asym υ, SO_2NH), 1152 (sym υ, SO_2NH), 1122, 1031 (δ, C-H), 633-558 cm^{-1} (υ, Fe-O).

✓ *SPIONs*-[*p*-(*N*-butylcarbamoyl)phenyl] Carbamate **14**



5 **14**

Scheme S3

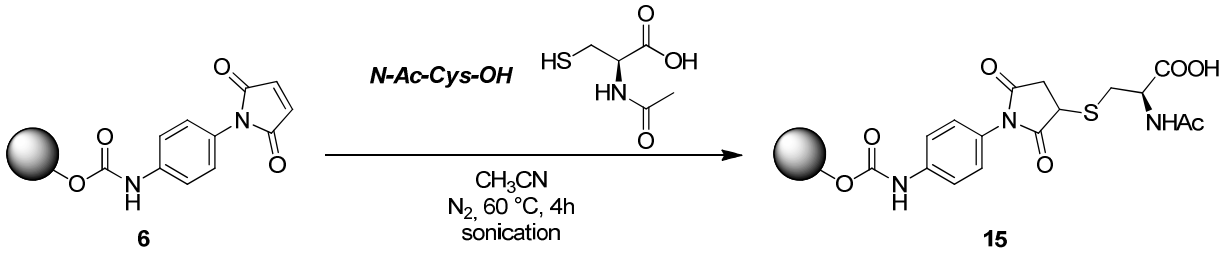
n-Butylamine (95 µl, 0.96 mmol, 5 eq) was added to a suspension of functionalized nanoparticles **5** (100 mg, loading 35.01% corresponding to 1.92 mmol/g_{NPs}, 1 eq) in dry $PhCH_3$ (5 ml), previously sonicated at 60 °C for 30 min; the mixture was then stirred, under sonication, at 60 °C for 4 h. After this time the suspension was centrifugated at 5000 r/min for 10 min and the supernatant was removed. The solid residue was washed with fresh $PhCH_3$ (3x5 ml), then with Et_2O (3x5 ml) and vortex/sonication/centrifugation were cyclic repeated for each washing. Functionalized NPs were dried on air overnight and then under vacuum for 24 h, to afford the nanoconjugates **14** as brown powders.

Elemental Analysis E.A. in CHN mode: for organic residue $C_{12}H_{15}N_2O_2$, found: C 28.40, H 2.97, N 5.52, O (calculated on molar nitrogen percentage) 6.30%.

Loadings from E.A.: 43.19%, corresponding to 1.97 mmol/g_{NPs}.

FTIR in trasmittance (blank KBr): 3324 (υ, N-H carbamate), 3193 (υ, N-H amide), 3037 (υ, ArC-H), 2962-2874 (υ, aliphatic C-H), 1678 (υ, C=O carbamate), 1649 (υ, C=O amide), 1596, 153o (υ, C=C), 1505 (δ, N-H), 1398, 1313, 1237 (υ, C-O and C-N), 1176 (δ, C-H), 632-577 cm^{-1} (υ, Fe-O).

✓ *SPIONs*-[*p*-(3-((*R*)-*N*-acetylcysteine)-2,5-dioxopyrrolidin-1-yl)phenyl] Carbamate **15**



Scheme S4

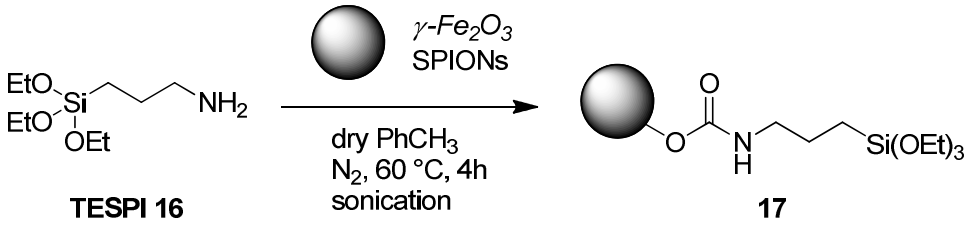
(*R*)-*N*-acetylcysteine (*N*-Ac-Cys-OH 78.66 mg, 0.482 mmol, 10 eq) was added to a suspension of functionalized nanoparticles **6** (20 mg, loading 51.81% corresponding to 2.41 mmol/g_{NPs}, 1 eq) in CH_3CN (5 ml), previously sonicated at 60 °C for 30 min; the mixture was then stirred, under sonication, at 60 °C for 4 h. After this time the suspension was centrifugated at 5000 r/min for 10 min and the supernatant was removed. The solid residue was washed with fresh CH_3CN (3x5 ml), then with Et_2O (3x5 ml) and vortex/sonication/centrifugation were cyclic repeated for each washing. Functionalized NPs were dried on air overnight and then under vacuum for 24 h, to afford the nanoconjugates **15** as brown powders.

Elemental Analysis E.A. in CHN mode: for organic residue $C_{16}H_{15}N_3O_6S$, found: C 32.77, H 2.78, N 6.12, O (calculated on molar nitrogen percentage) 13.98, S (calculated on molar nitrogen percentage) 4.67%.

Loadings from E.A.: 60.32%, corresponding to 1.60 mmol/g_{NPs}.

FTIR in trasmittance (blank KBr): 3360 (broad υ, CO_2H and N-H), 3073 (υ, ArC-H), 2970-2923 (υ, aliphatic C-H), 1777 (υ, C=O amide), 1709 (υ, C=O imide), 1692 (shoulder, C=O carbamate, amide and carboxylic acid), 1604, 1549 (δ, N-H), 1512 (υ, C=C), 1393 (υ, Ar-N), 1314 (υ, C-N imide), 1233-1180 (υ, C-O and C-N), 1175 (shoulder δ, C-H), 834 (δ, C-H out of plane), 689-585 cm^{-1} (υ, Fe-O).

✓ *SPIONs*-(*N*-(3-Triethoxysilyl)propyl) Carbamate **17**



Scheme S5

Naked SPIONs (100 mg) were suspended in dry toluene (5 ml) and sonicated for 30 min at 60 °C. To this suspension was then added the 3-(triethoxysylil)propyl isocyanate (TESPI **17**) (0.8 mmol) and the mixture was sonicated for 4 h at 60 °C. After this time, the suspension was centrifugated at 5000 r/min for 10 min and the supernatant was removed. The solid residue was washed with fresh $PhCH_3$ (3x5 ml), then with Et_2O (3x5 ml) and vortex/sonication/centrifugation were cyclic repeated for each washing. Functionalized NPs

were dried on air overnight and then under vacuum for 24 h, to afford the corresponding nanoconjugate as brown powders.

Elemental Analysis E.A. in CHN mode: for organic residue $C_{10}H_{22}NO_4Si$, found: C 4.99, H 1.71, N 0.74, O (calculated on molar nitrogen percentage) 3.38, Si (calculated on molar nitrogen percentage) 1.48%.

Loadings from E.A.: 12.30%, corresponding to 0.50 mmol/g_{NPs}.

FTIR in trasmittance (blank KBr): 3335 (broad υ, N-H and Fe-OH), 2974-2885 (υ, aliphatic C-H), 1635 (υ, C=O carbamate), 1566 (δ, N-H), 1443, 1274, 1103-1078 (υ, Si-O), 1032, 955, 685-583 cm^{-1} (υ, Fe-O).

III. Elemental analysis and determination of the loading

All of the nanoconjugates **1-6**, **13-15** and **17** were submitted to E.A., from which the chemical formula of the organic residues anchored onto the SPIONs surface was confirmed. From the carbon, hydrogen and nitrogen percentage was therefore possible to determine the quantity of organic residue anchored onto SPIONs. This total percentage of organic part [***Tot % org***] corresponds to the quantity in milligrams of the organic residue *per* 100 milligrams of nanoconjugates. From the molecular weight of the anchored molecule is therefore possible to express the final loading as millimoles of organic part *per* gram of functionalized nanoparticles [***mmol/g*** $_{NPs}$].

In order to verify the possibility of modulating the loading of the isocyanates onto the SPIONs, three sets of experiments were performed, using increasing amounts of compounds **7-12** (0.2, 0.4 or 0.8 mmoles) in reaction with 100 mg of naked NPs.

The values obtained are resumed in *Table S1, Table S2* and *Table S3*, and a graphical comparison was also shown in *Figure S1* and *Figure S2*.

Table S1 *- Final loading for nanoconjugates* **1-6** *in the reaction with 0.2 millimoles of isocyanate per 100 milligrams of naked SPIONs.*

R-NCO	***Nanoconjugate***	**0.2 mmol of NCO / 100 mg NPs**	
		Tot % org	*mmol/g*$_{NPs}$
7	**1**	13.83	1.10
8	**2**	15.36	1.28
9	**3**	16.62	1.08
10	**4**	17.54	0.80
11	**5**	22.17	1.21
12	**6**	14.42	0.67
Average Loading		***16.66***	***1.02***

Table S2 *- Final loading for nanoconjugates* **1-6** *in the reaction with 0.4 millimoles of isocyanate per 100 milligrams of naked SPIONs.*

R-NCO	***Nanoconjugate***	**0.4 mmol of NCO / 100 mg NPs**	
		Tot % org	*mmol/g_{NPs}*
7	**1**	26.69	2.11
8	**2**	26.55	2.21
9	**3**	29.83	1.93
10	**4**	19.74	0.90
11	**5**	25.74	1.41
12	**6**	31.07	1.44
Average Loading		***26.60***	***1.67***

Table S3 *- Final loading for nanoconjugates* **1-6** *in the reaction with 0.8 millimoles of isocyanate per 100 milligrams of naked SPIONs.*

R-NCO	***Nanoconjugate***	**0.8 mmol of NCO / 100 mg NPs**	
		Tot % org	*mmol/g_{NPs}*
7	**1**	51.47	4.08
8	**2**	43.80	3.65
9	**3**	40.33	2.61
10	**4**	21.75	0.99
11	**5**	35.01	1.92
12	**6**	51.81	2.41
Average Loading		***40.70***	***2.61***

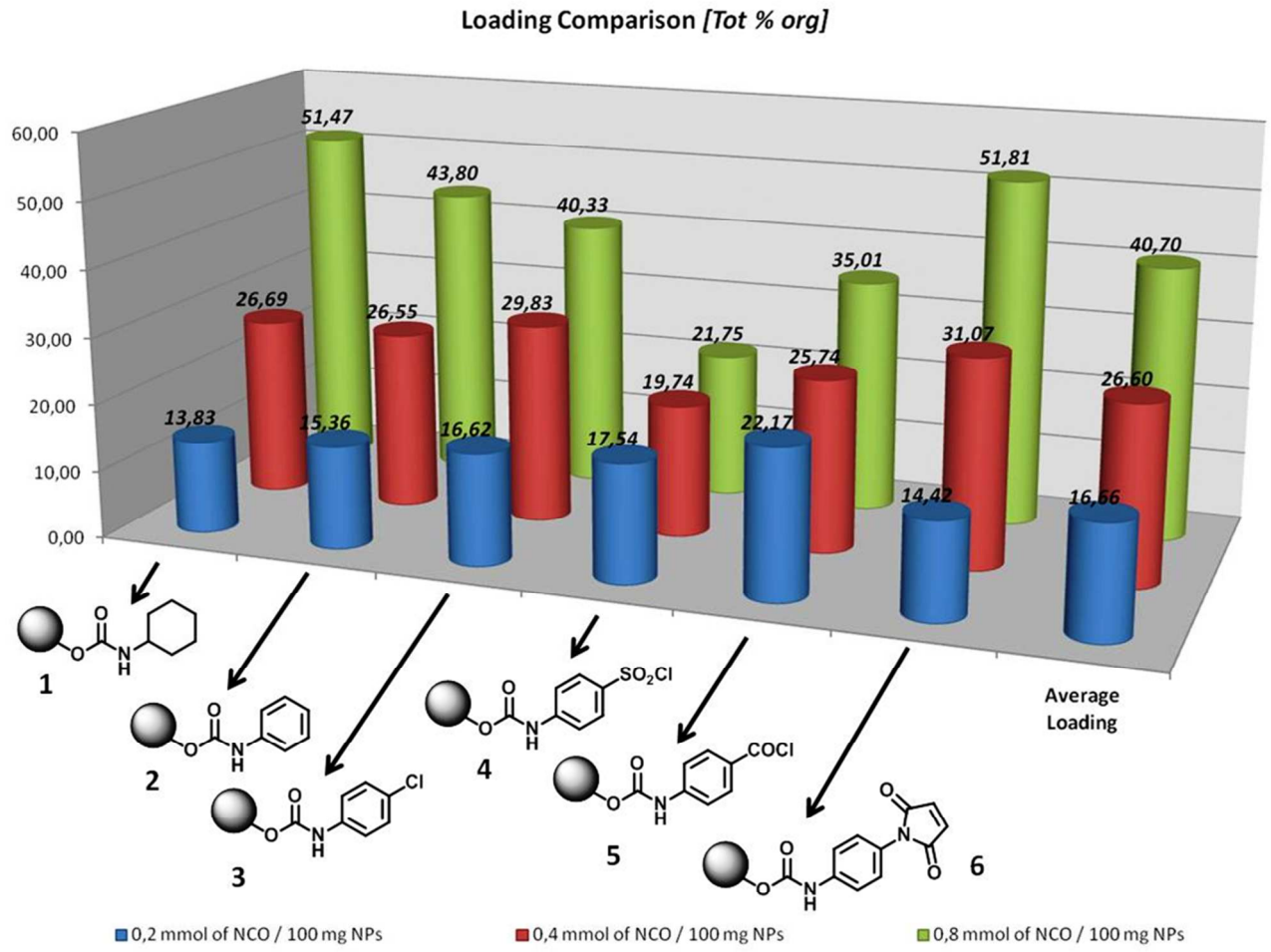


Figure S1 *- Loading comparison of nanoconjugates* **1-6**, *express as total percentage in weight of organic part loaded onto SPIONs (Tot % org).*

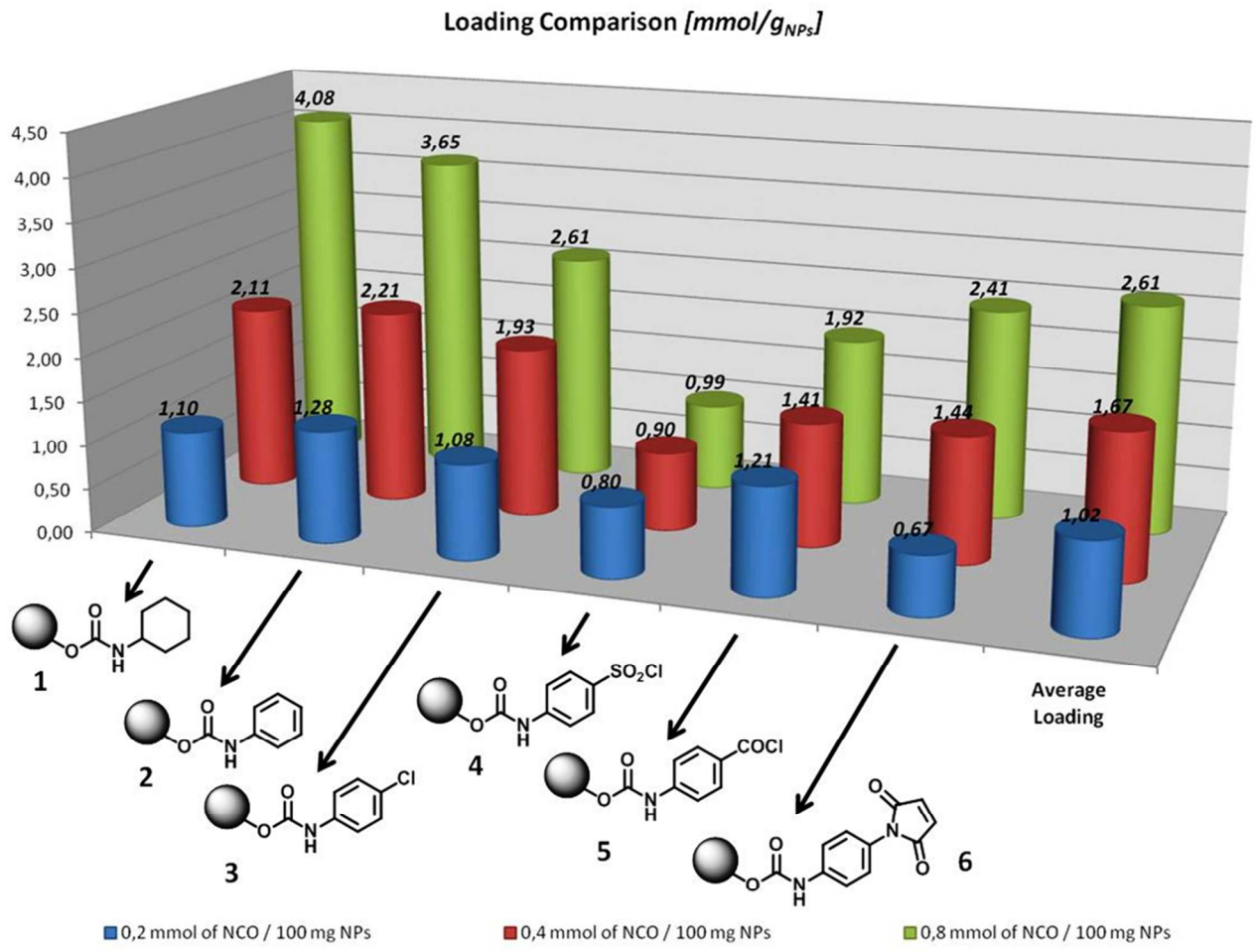


Figure S2 *- Loading comparison of nanoconjugates* **1-6***, express as millimoles of organic part per gram of functionalized nanoparticles (mmol/g$_{NPs}$).*

The reproducibility of the reactions of isocyanate with SPIONs was confirmed in the case of PMPI **12**. In fact, three experiments in which 0.8 millimoles of **12** were reacted with 100 milligrams of naked SPIONs, afforded the nanoconjugate **6** with approximately the same loading values (*Table 4*).

Table S4 *- Reproducibility of the loading in the synthesis of nanoconjugate* **6**.

Entry	**0.8 mmol of PMPI / 100 mg NPs**	
	Tot % org	*mmol/g_{NPs}*
A	51.81	2.41
B	52.12	2.42
C	51.11	2.38

In the graph reported in *Figure S3*, the average loadings expressed in [*Tot % org*] and [*mmol/g_{NPs}*] are reported with their linear regression trend lines, demonstrating the possibility to easy tune the loading of the desired nanoconjugate.

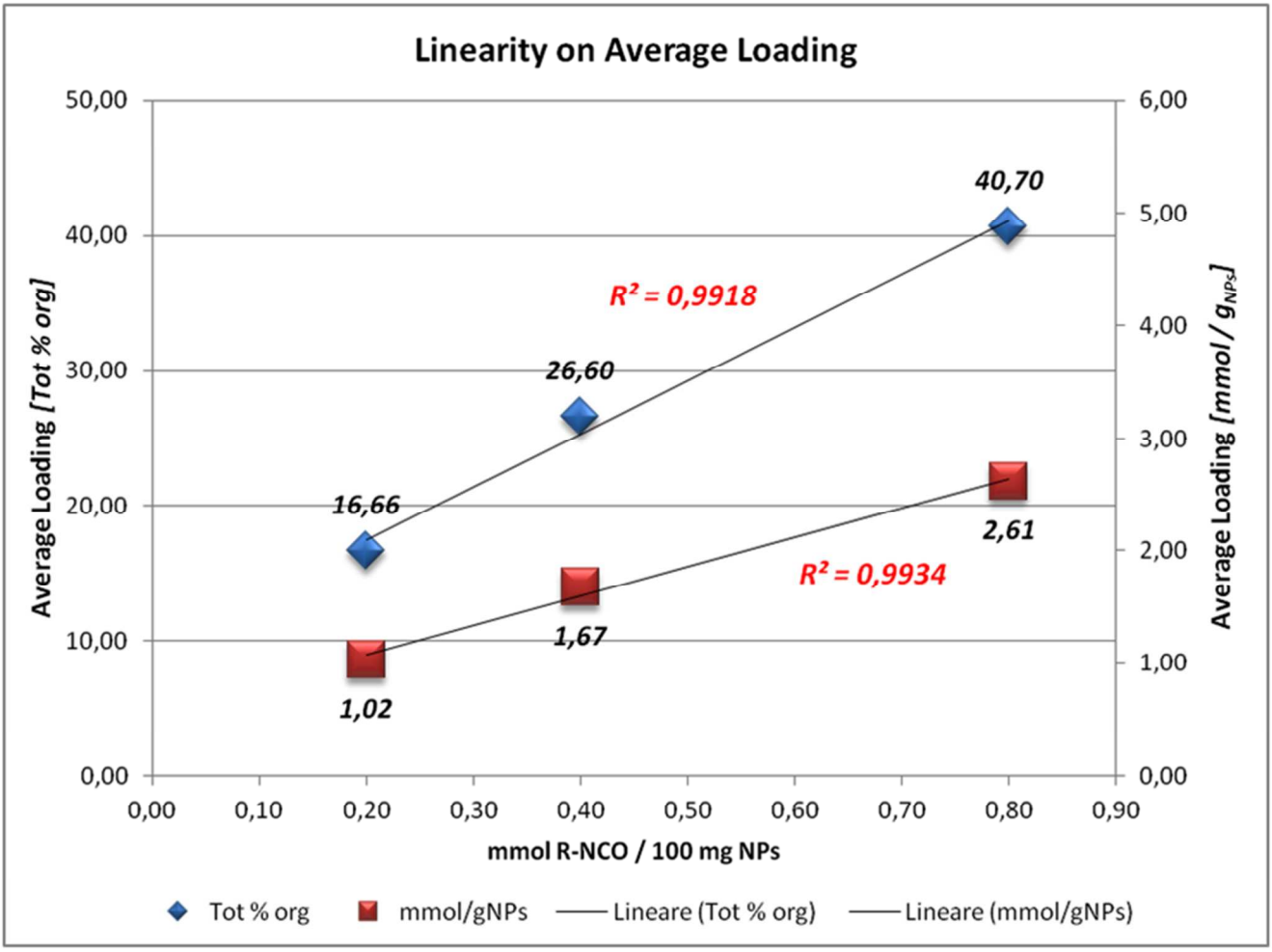


Figure 3 *- Linear regressions on average loading values.*

IV. FTIR spectroscopy

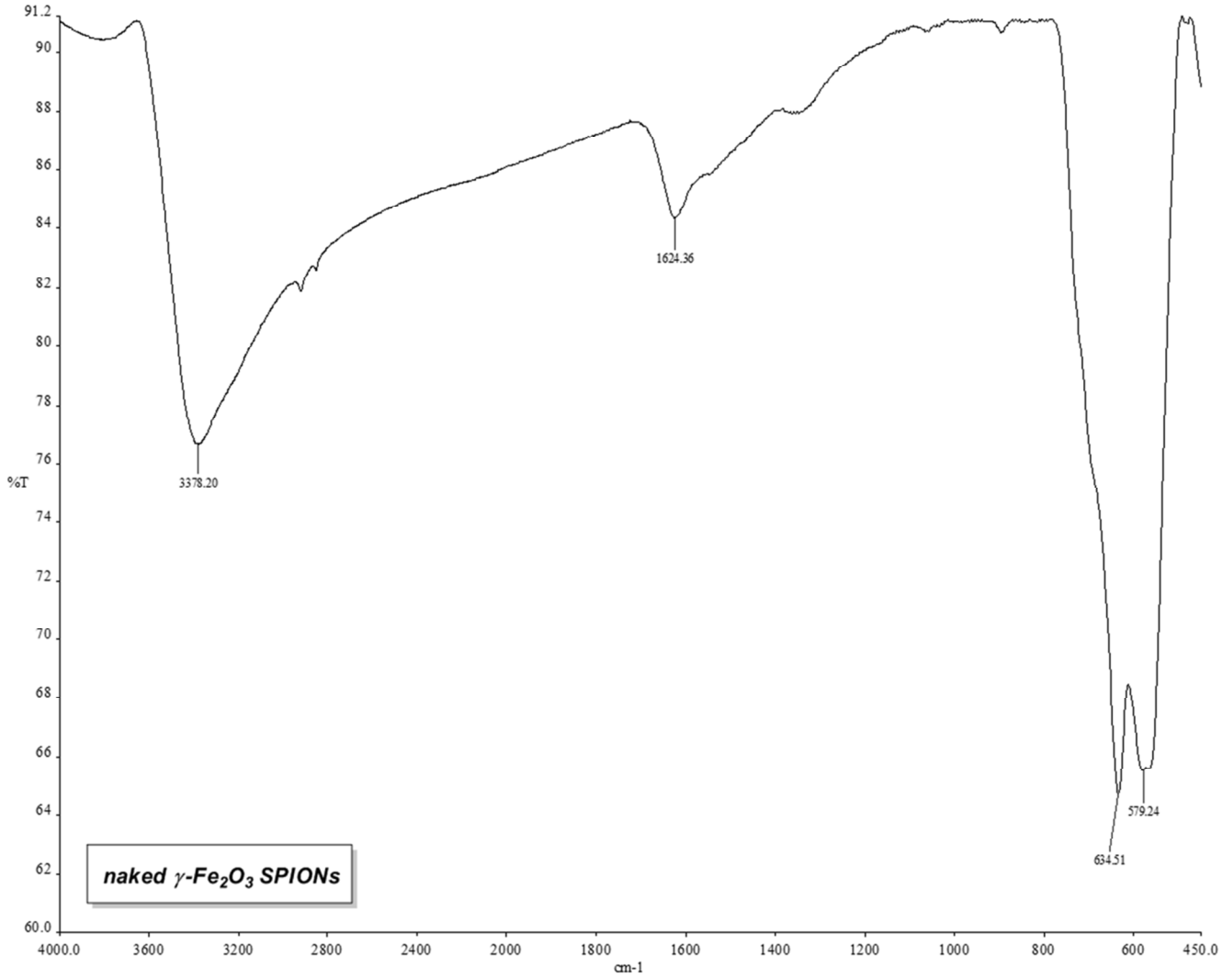


Figure S4 *- FTIR spectrum of naked commercially available SPIONs.*

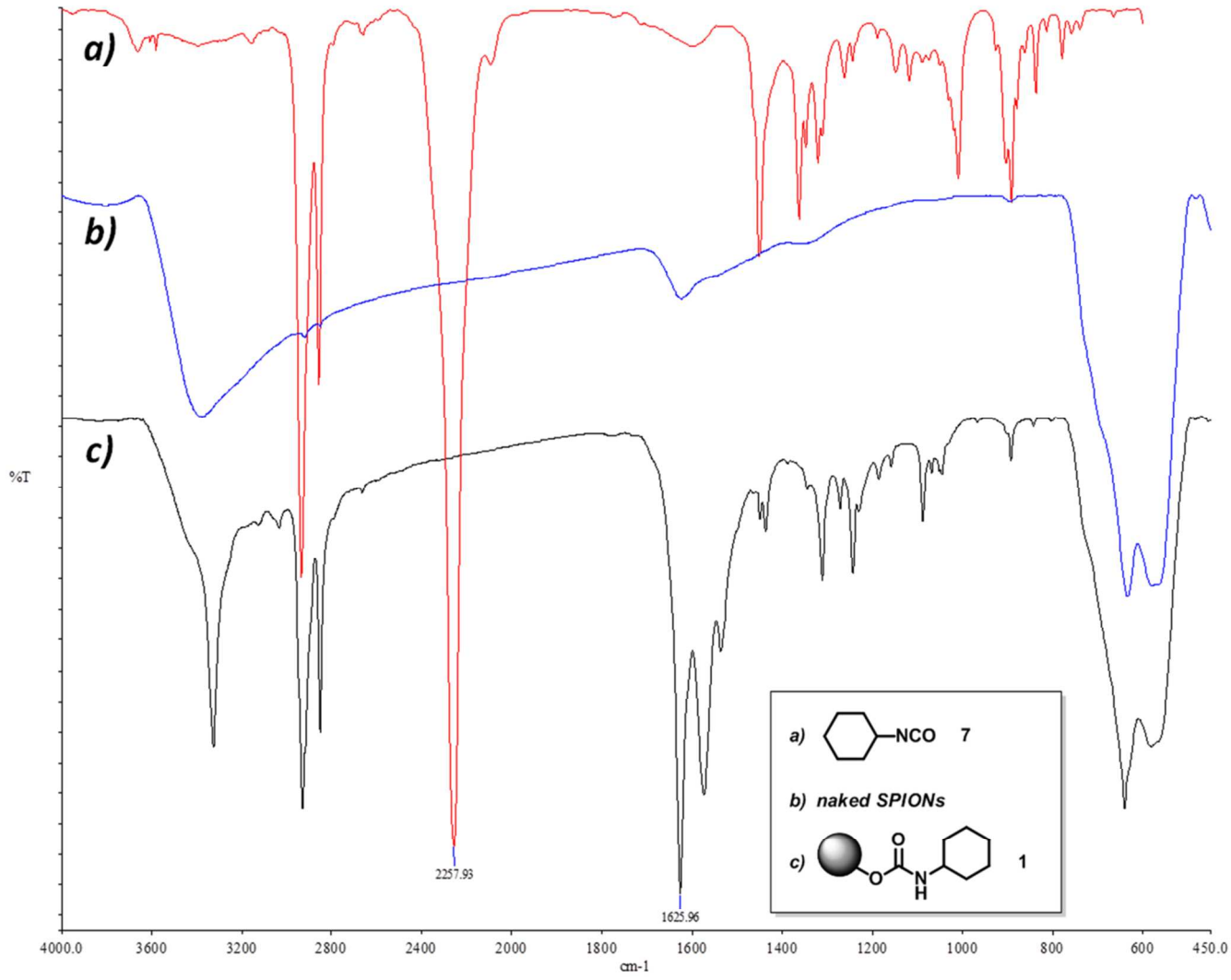


Figure S5 *- FTIR spectra of a) cyclohexyl isocyanate* **7** *(red line), b) naked SPIONs (blue line) and c) nanoconjugate* **1** *(black line). The labeled peak at 2258 cm^{-1} in spectrum a), corresponding to NCO stretching, is no longer visible in spectrum c), in which are instead clearly visible all the vibrational bands related to the C-H bonds and at 1626 cm^{-1} the sharp peak of carbamate C=O stretching. At about 3300 cm^{-1}, the stretching of carbamate N-H bond is also present.*

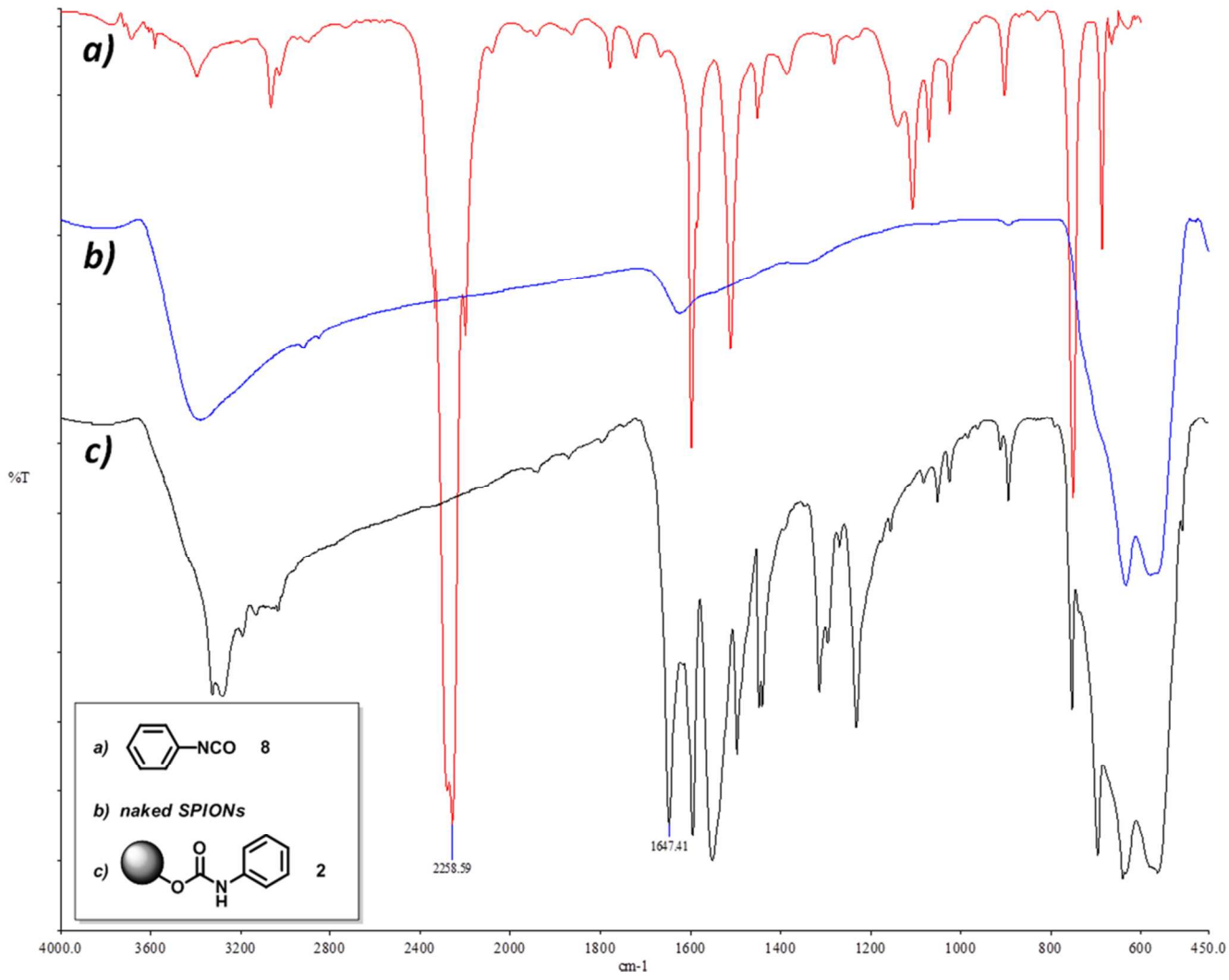


Figure S6 *- FTIR spectra of a) phenyl isocyanate* **8** *(red line), b) naked SPIONs (blue line) and c) nanoconjugate* **2** *(black line). The labeled peak at 2259 cm^{-1} in spectrum a), corresponding to NCO stretching, is no longer visible in spectrum c), in which are instead clearly visible all the vibrational bands related to the C-H and C=C bonds and at 1647 cm^{-1} the sharp peak of carbamate C=O stretching. At about 3300 cm^{-1}, the stretching of carbamate N-H bond is also present.*

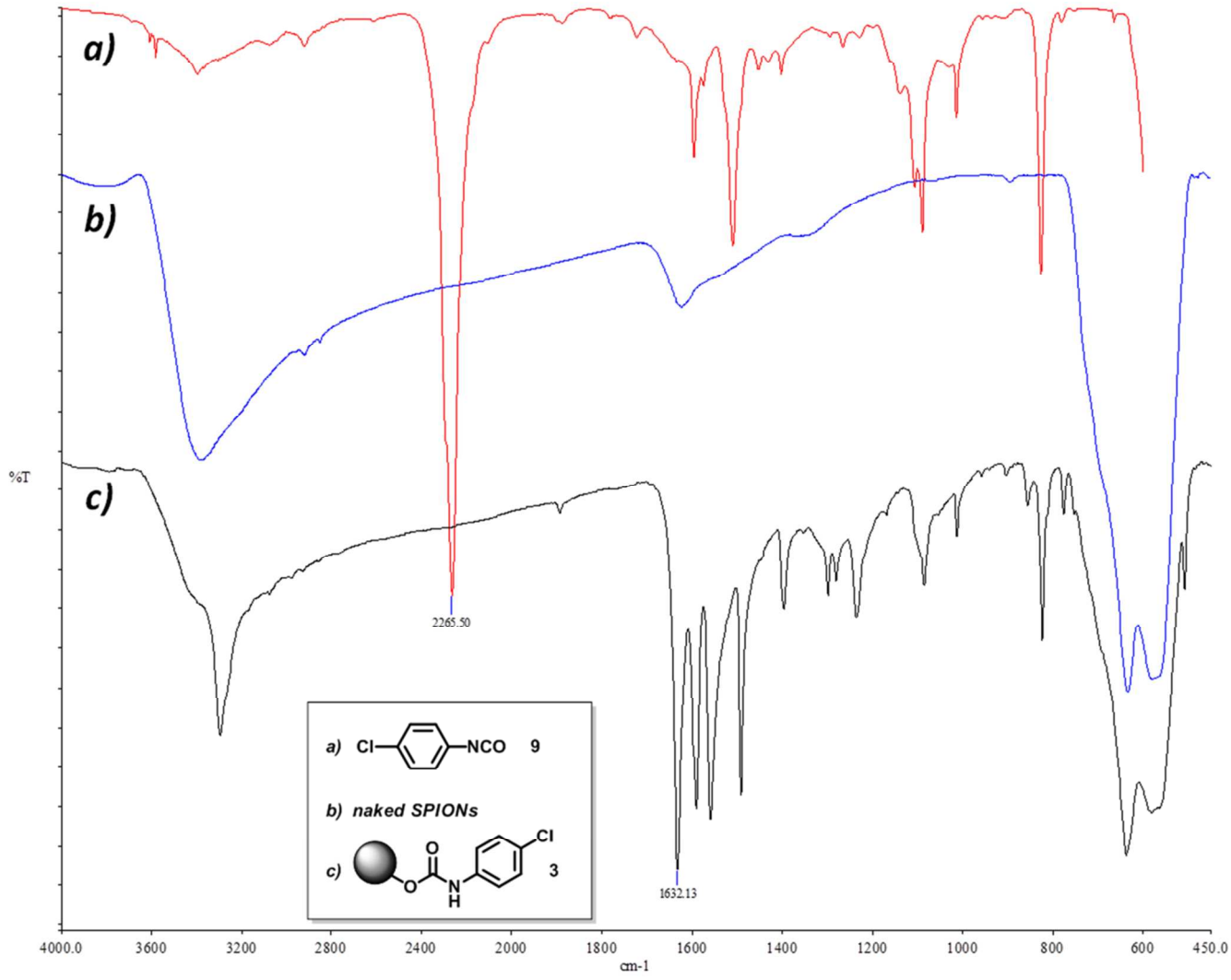


Figure S7 *- FTIR spectra of a) p-chlorophenyl isocyanate* **9** *(red line), b) naked SPIONs (blue line) and c) nanoconjugate* **3** *(black line). The labeled peak at 2265 cm^{-1} in spectrum a), corresponding to NCO stretching, is no longer visible in spectrum c), in which are instead clearly visible all the vibrational bands related to the C-H and C=C bonds and at 1632 cm^{-1} the sharp peak of carbamate C=O stretching. At about 3300 cm^{-1}, the stretching of carbamate N-H bond is also present.*

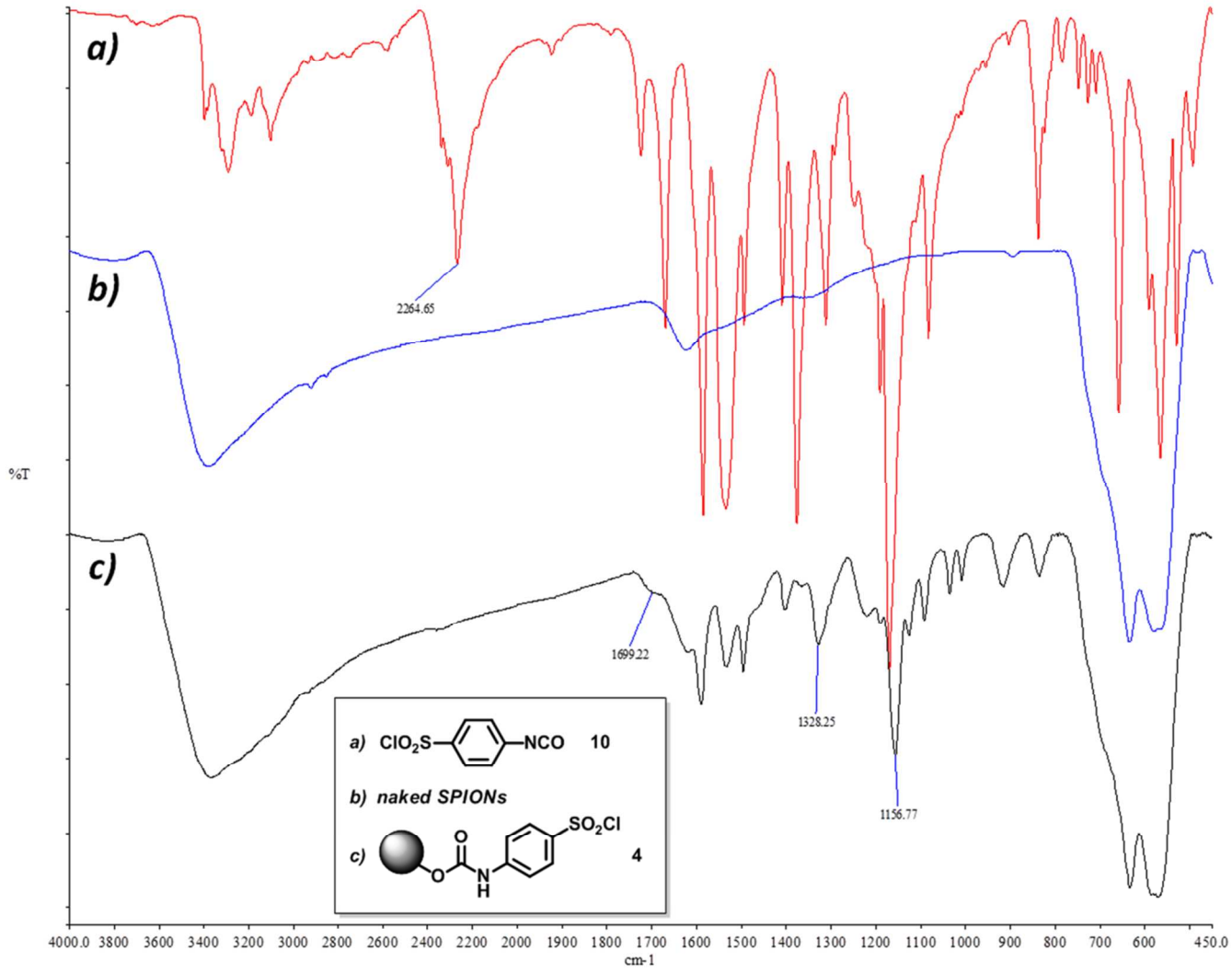


Figure S8 *- FTIR spectra of a) (p-chlorosulfonyl)phenyl isocyanate* **10** *(red line), b) naked SPIONs (blue line) and c) nanoconjugate* **4** *(black line). The labeled peak at 2265 cm^{-1} in spectrum a), corresponding to NCO stretching, is no longer visible in spectrum c), in which are instead clearly visible all the vibrational bands related to the C-H and C=C bonds, the classical stretching of O=S=O moiety at 1328-1157 cm^{-1} and at 1699 cm^{-1} the shouldered peak of carbamate C=O stretching.*

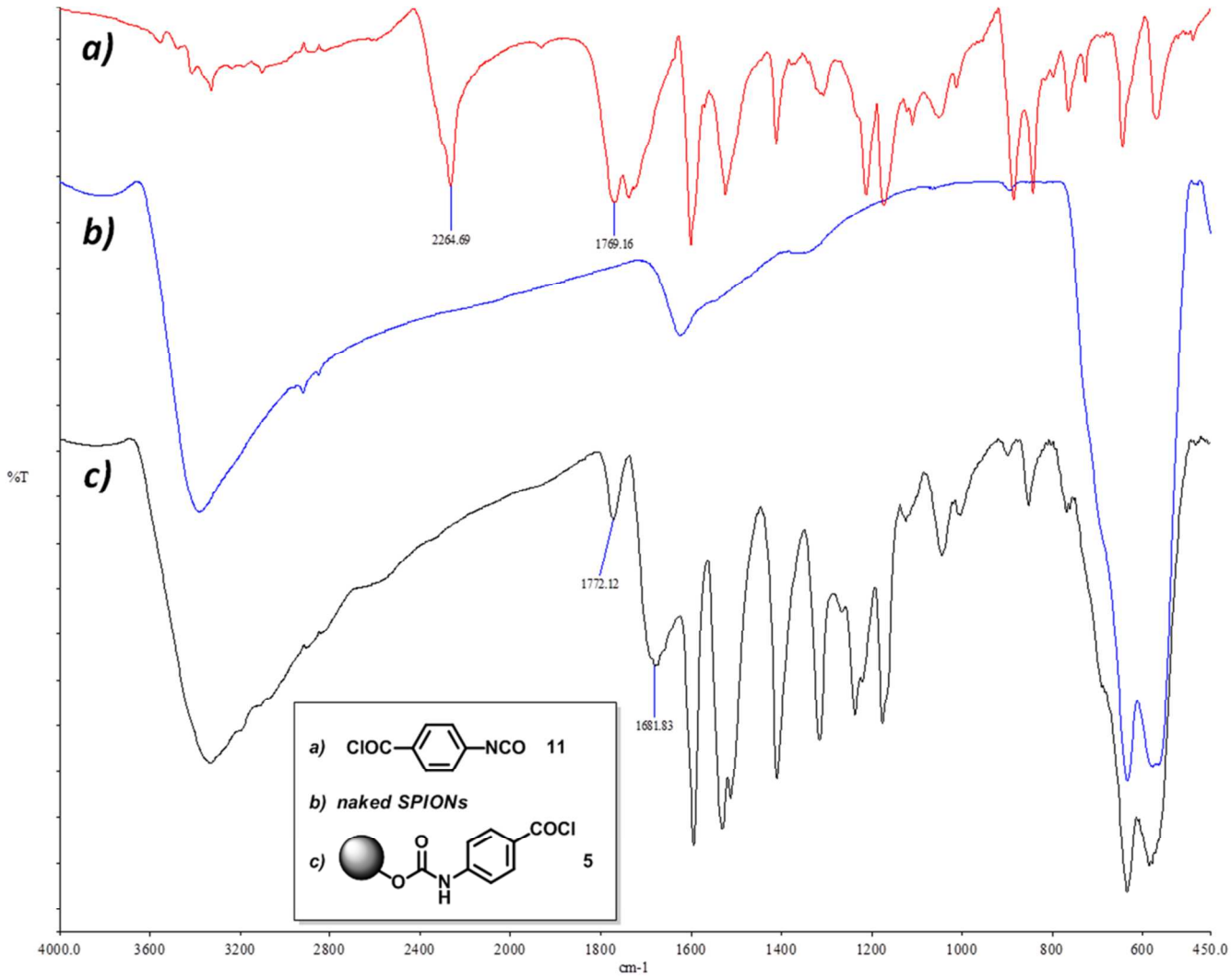


Figure S9 *- FTIR spectra of a) (p-isocyanate)benzoyl chloride* **11** *(red line), b) naked SPIONs (blue line) and c) nanoconjugate* **5** *(black line). The labeled peak at 2265 cm^{-1} in spectrum a), corresponding to NCO stretching, is no longer visible in spectrum c), in which are instead clearly visible all the vibrational bands related to the C-H and C=C bonds, the classical stretching of chloroacyl moiety at about 1770 cm^{-1} and at 1682 cm^{-1} the peak of carbamate C=O stretching.*

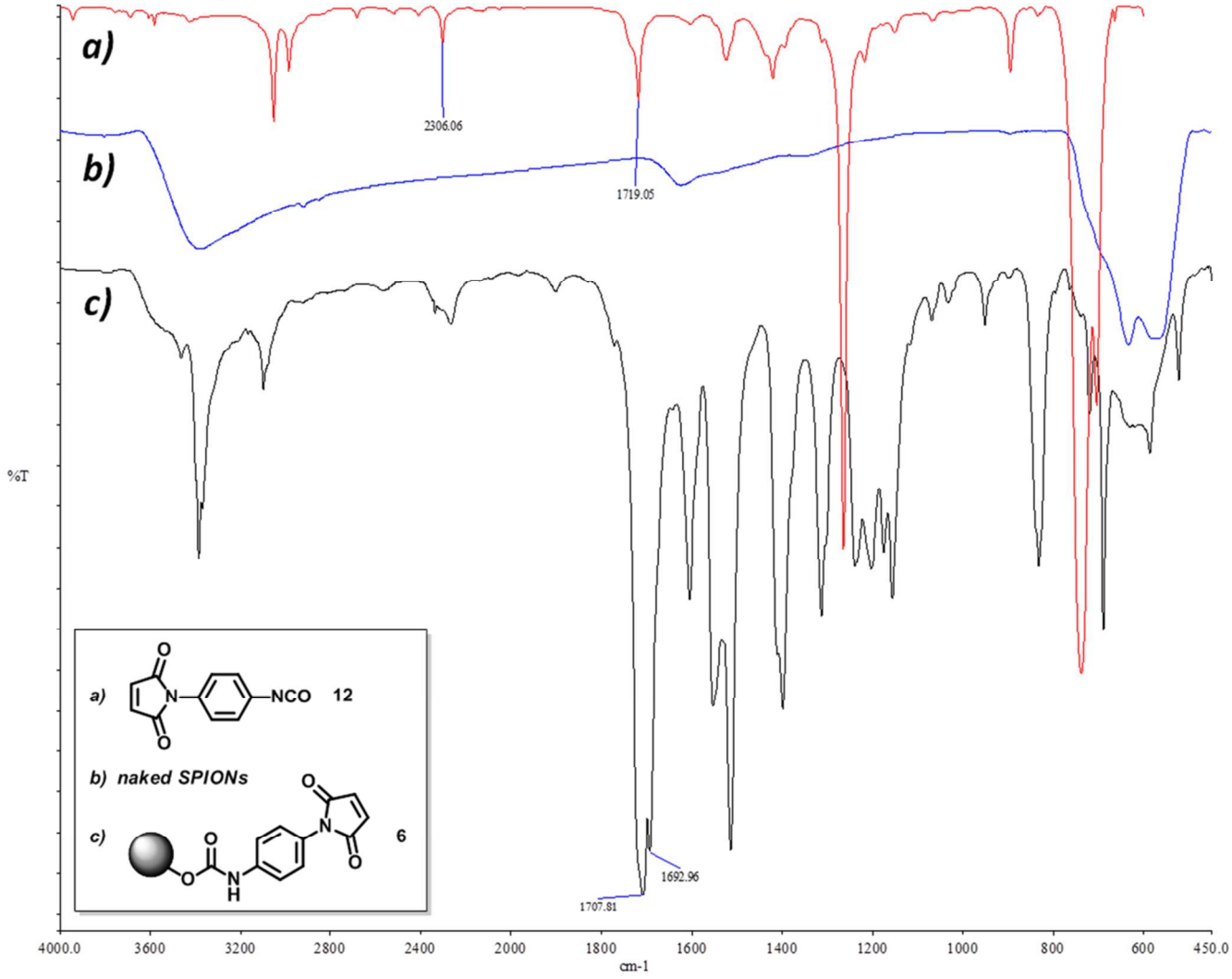


Figure S10 *- FTIR spectra of a) (p-maleimido)phenyl isocyanate PMPI* **12** *(red line), b) naked SPIONs (blue line) and c) nanoconjugate* **6** *(black line). The labeled peak at 2306 cm^{-1} in spectrum a), corresponding to NCO stretching, is no longer visible in spectrum c), in which are instead clearly visible all the vibrational bands related to the C-H and C=C bonds, the maleimido C=O stretching peak at about 1710 cm^{-1} and at 1693 cm^{-1} the sharp peak of carbamate C=O stretching. At about 3300 cm^{-1}, the stretching of carbamate N-H bond is also present.*

Table S5 *- C=O Stretching values of starting isocyanate* **7-12** *and corresponding nanoconjugates* **1-6**.

R-NCO	***Nanoconjugate***	***Stretching [cm^{-1}]***		***Figure***
		-N=C=O	*C=O carbamate*	
7	**1**	2258	1626	S5
8	**2**	2259	1647	S6
9	**3**	2265	1632	S7
10	**4**	2265	1699	S8
11	**5**	2265	1682	S9
12	**6**	2306	1693	S10

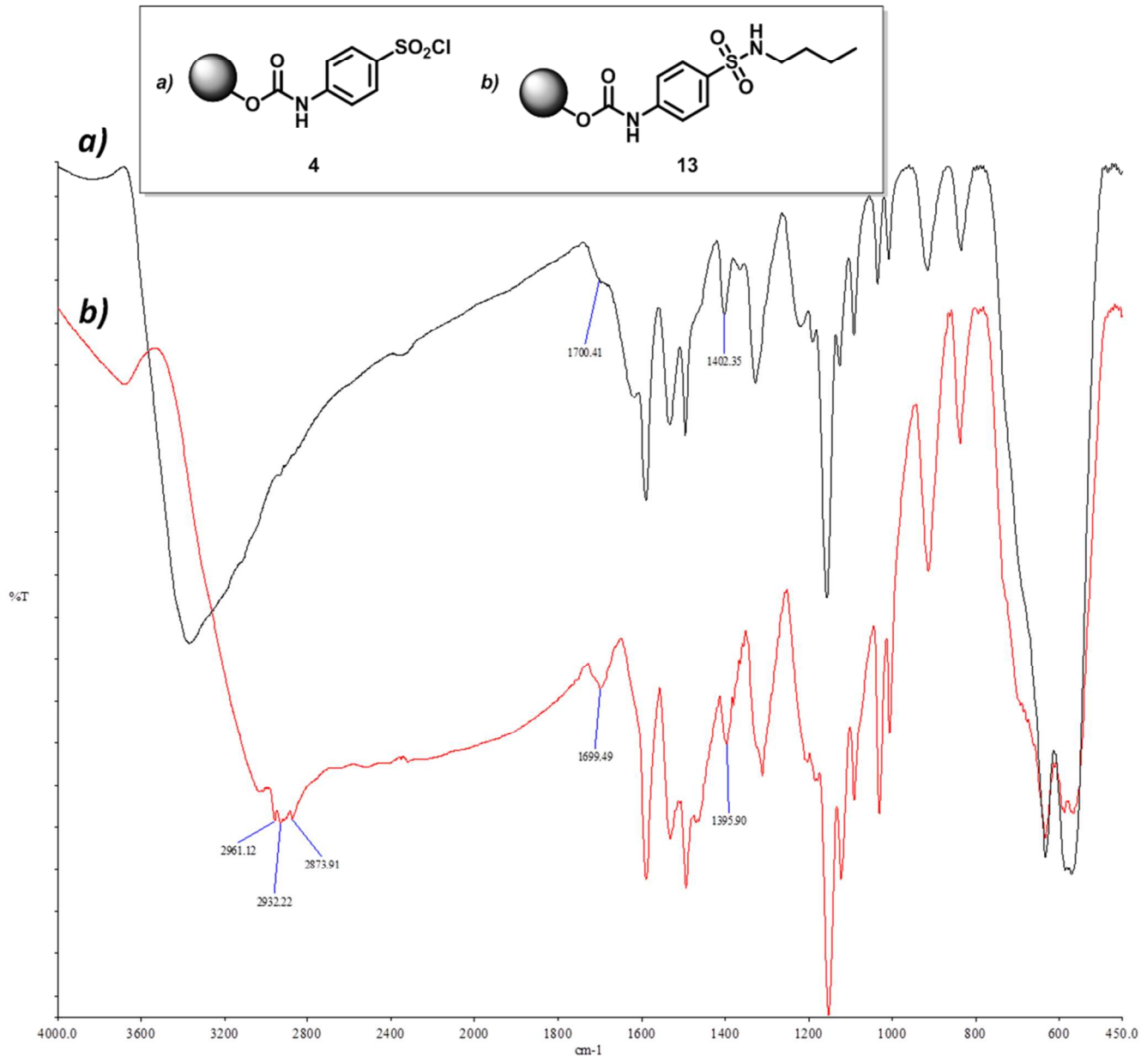


Figure S11 *- FTIR spectra of a) nanoconjugate* **4** *(black line) and b) nanoconjugate* **13** *(red line). In spectrum b) are clearly visible the stretchings at high wavenumber related to C-H bonds of the butyl chain. It's also clearly visible the C=O band of the carbamate and a significant modification in the vibrational bands in the range of 1600-1400 cm^{-1} referred to the presence of the sulfonamide bond and to the bending of N-H and C-H bonds.*

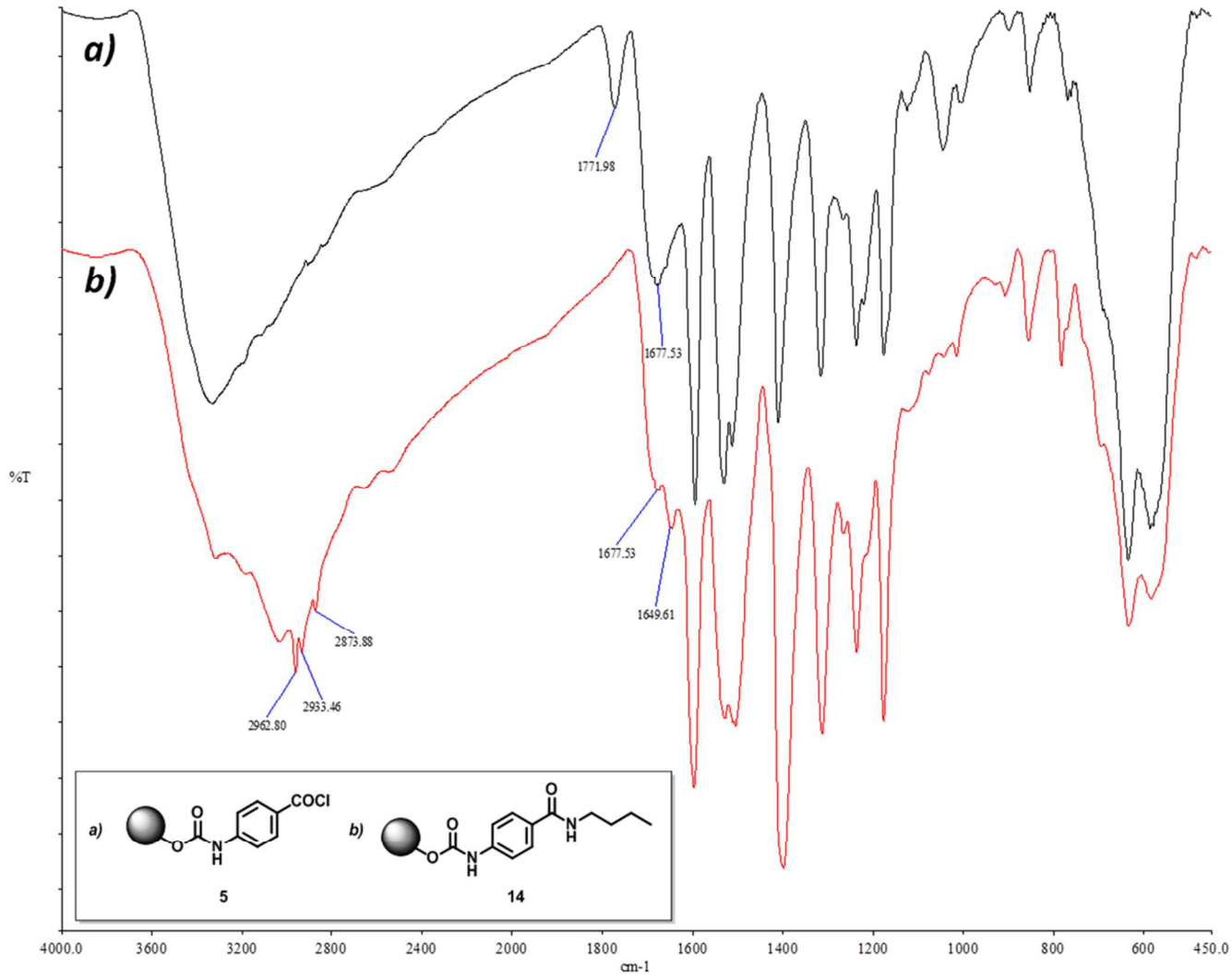


Figure S12 *- FTIR spectra of a) nanoconjugate* **5** *(black line) and b) nanoconjugate* **14** *(red line). In spectrum b) are clearly visible the stretchings at high wavenumber related to C-H bonds of the butyl chain. Are also clearly visible the C=O band of the carbamate (1677 cm^{-1}) and the stretching of the C=O amide moiety (1649 cm^{-1}), while the peak related to the Cl-C=O at 1772 cm^{-1} in spectrum a) disappeared in spectrum b).*

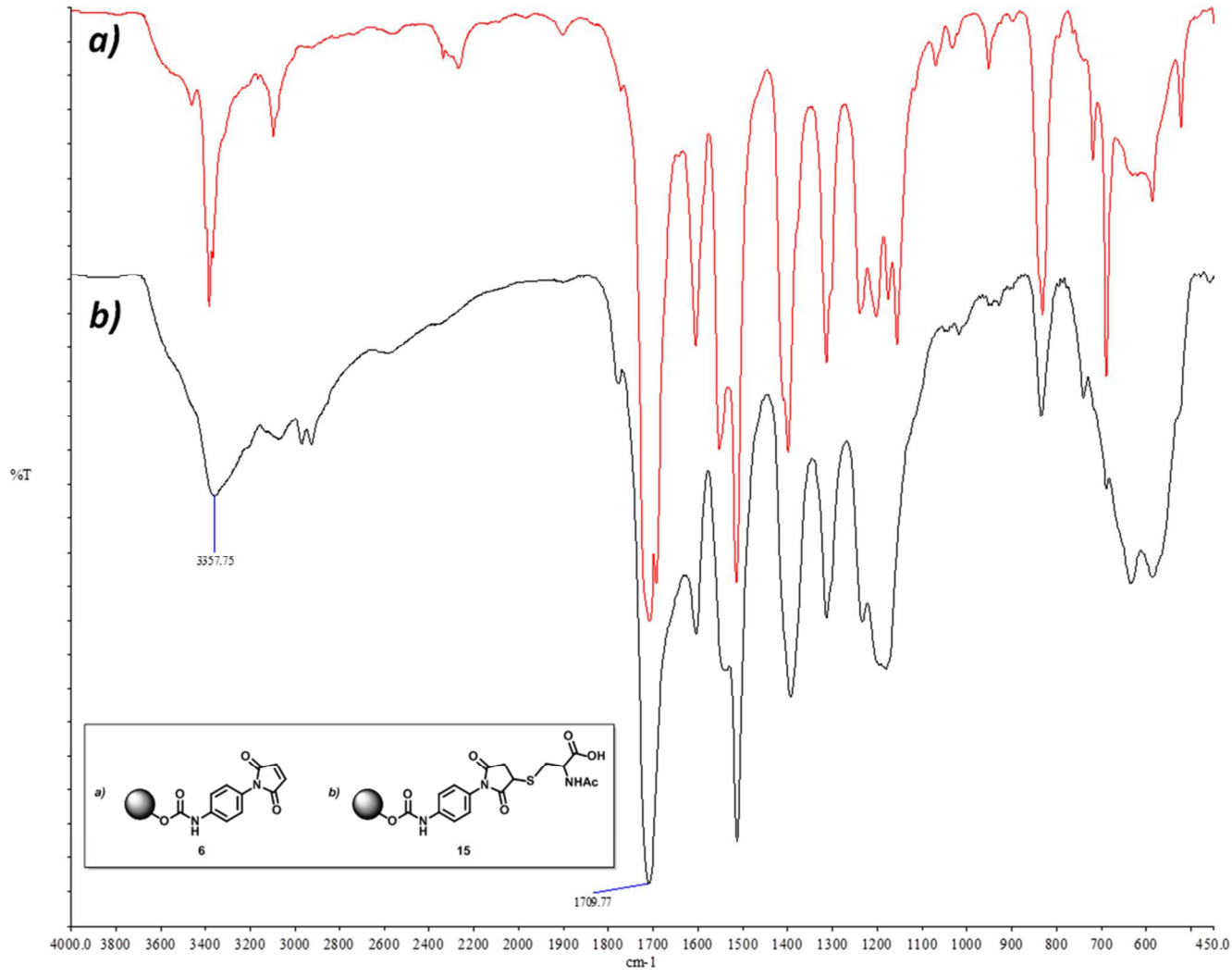


Figure S13 *- FTIR spectra of a) nanoconjugate* **6** *(red line) and b) nanoconjugate* **15** *(black line). In spectrum b) is clearly visible the absorption peak at about 3360 cm^{-1} typical of OH group in carboxylic acid. The broad band centered at 1709 cm^{-1} is referred to the presence of all C=O stretchings (maleimido, carbamate, amide and carboxylic moieties).*

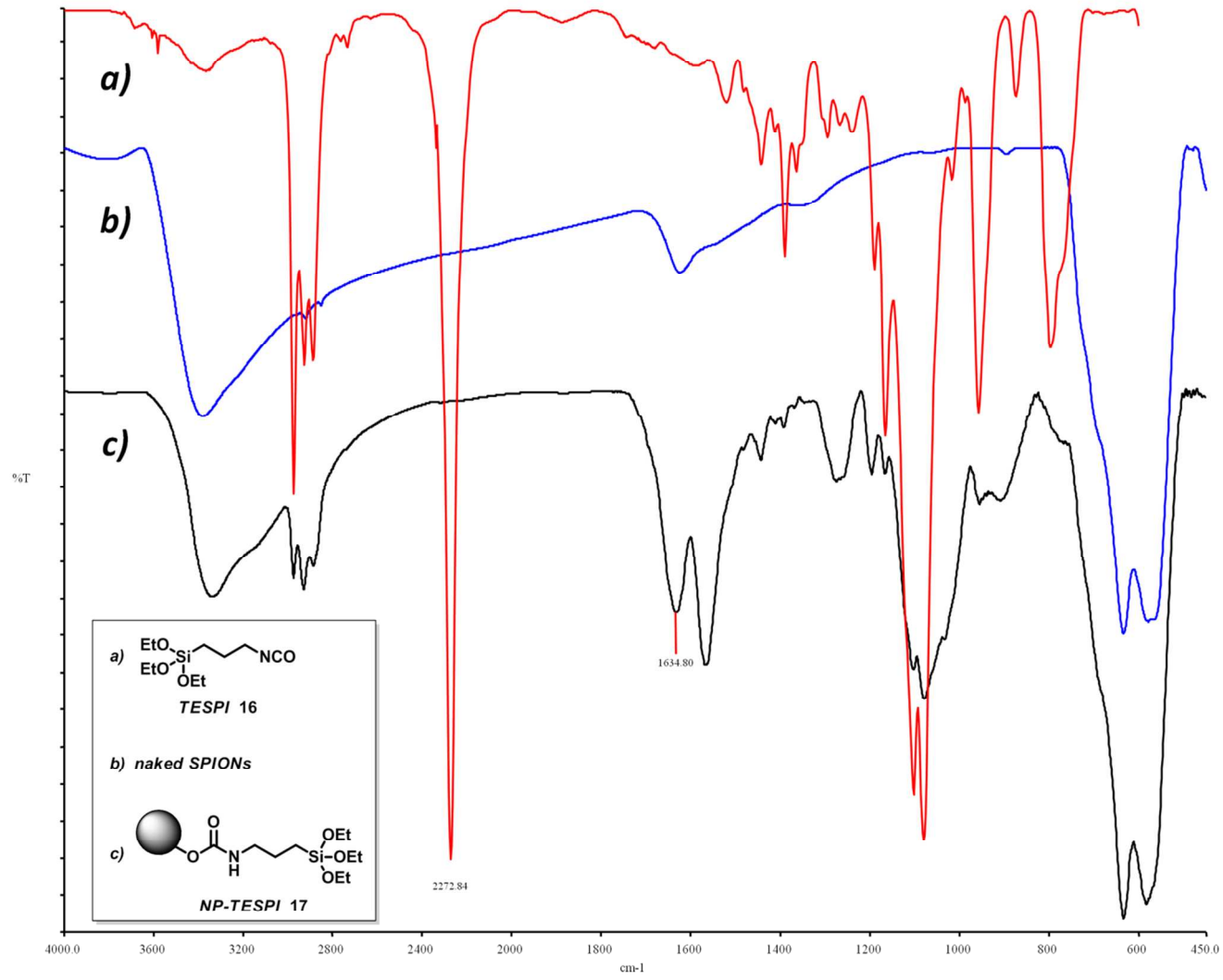


Figure S14 *- FTIR spectra of a) triethoxysilylpropyl isocyanate TESPI* **16** *(red line), b) naked SPIONs (blue line) and c) nanoconjugate* ***17*** *(black line). The labeled peak at 2273 cm^{-1} in spectrum a), corresponding to NCO stretching, is no longer visible in spectrum c), in which are instead clearly visible all the vibrational bands related to the C-H bonds (2974-2885 cm^{-1}) and at 1635 cm^{-1} the peak of carbamate C=O stretching. At about 1103-1078 cm^{-1}, the vibrational bands of Si-O bonds are also present.*

V. HR-MAS NMR spectroscopy

The High Resolution Magic Angle Spinning (HR-MAS) probes have been designed to perform solution type experiments, while spinning the sample at the magic angle (54.7°) and are able to work with gel samples and semisolid matrices; they have been used successfully for the characterization of gels,[ii] biopsies,[iii] nanocrystalline proteins,[iv] metabolic phenotypes of entire microorganisms[v] and also with intact tissues, with a spectral resolution comparable to that observed with extract solutions.[vi]

As just described in previous works, ^{1}H-NMR resolved spectra of a ligands bound to a paramagnetic nanocrystal, like SPIONs, are difficult to perform for many reasons: the large broadening effects caused by the paramagnetic material,[vii] that also measurably changes the nuclear magnetic resonance relaxation properties of nearby protons in aqueous solution, at distances up to ca. 50 μm;[viii] the decreased mobility of the ligands on the surface, as well as the paramagnetism of the iron oxide, in addition to an inherent broadness in the spectra, causes the lack of splitting in the peaks and, in order to obtain a ^{1}H-NMR spectrum with conventional NMR probes, with a minimal resolution, the solution has to be extremely dilute.[ix]

Working in this direction, some ^{1}H spectra were initially reported with a quite good signal shape[vii,ix] but the resolution was still not enough to clearly show the multiplet's structure, also with very diluted and simple molecules.

By the way, it was shown[x] that the HR-MAS NMR technique, could be a powerful tool to overcome problems such as the low resolution of the ^{1}H spectra not only against the chemical shift anisotropy problem, but also decreasing the paramagnetic effects, allowing the characterization of the structures of different organic ligands bound to superparamagnetic iron oxide nanoparticles (both Fe_2O_3 or Fe_3O_4 SPIONs) producing resolved ^{1}H spectra with structured NMR signals.

We have just effectively used this NMR technique in a precedent work[xi] analyzing Peptide Nucleic Acid (PNA) strands linked on magnetic maghemite nanoparticles and producing sets of ^{1}H resolved spectra.

In this work we have applied again this analytic tool performing different kind of NMR experiments, in order to characterize some of the obtained nanoconjugates and, at the same time, with the aim to give an additional prove of the carbamate bond formation.

In *Table S6*, the HR-MAS experiments performed on nanoconjugates **1-3** and **6** are summarized.

Table S6 *- HR-MAS experiments performed on some nanoconjugates.*

Nanoconjugate	**HR-MAS experiments**
1	^{1}H, ^{13}C, COSY, HSQC
2	^{1}H, ^{13}C
3	^{1}H, ^{13}C
6	^{1}H, ^{13}C

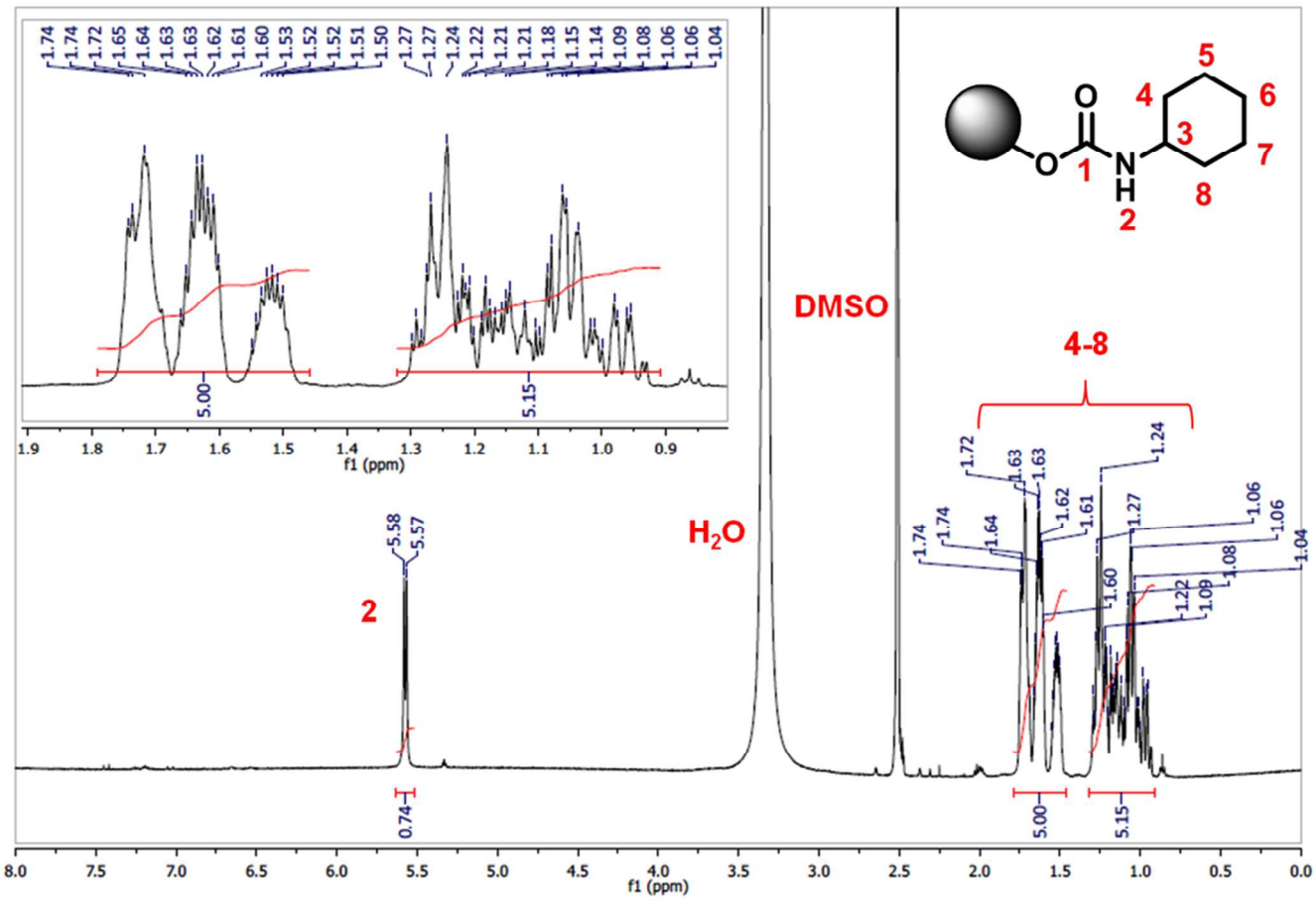


Figure S15 - *^{1}H HR-MAS NMR spectrum of nanoconjugate* **1** *in DMSO (4 KHz speed). Signal of proton 3 (CH) lays under water peak; attribution were done by bidimensional experiments.*

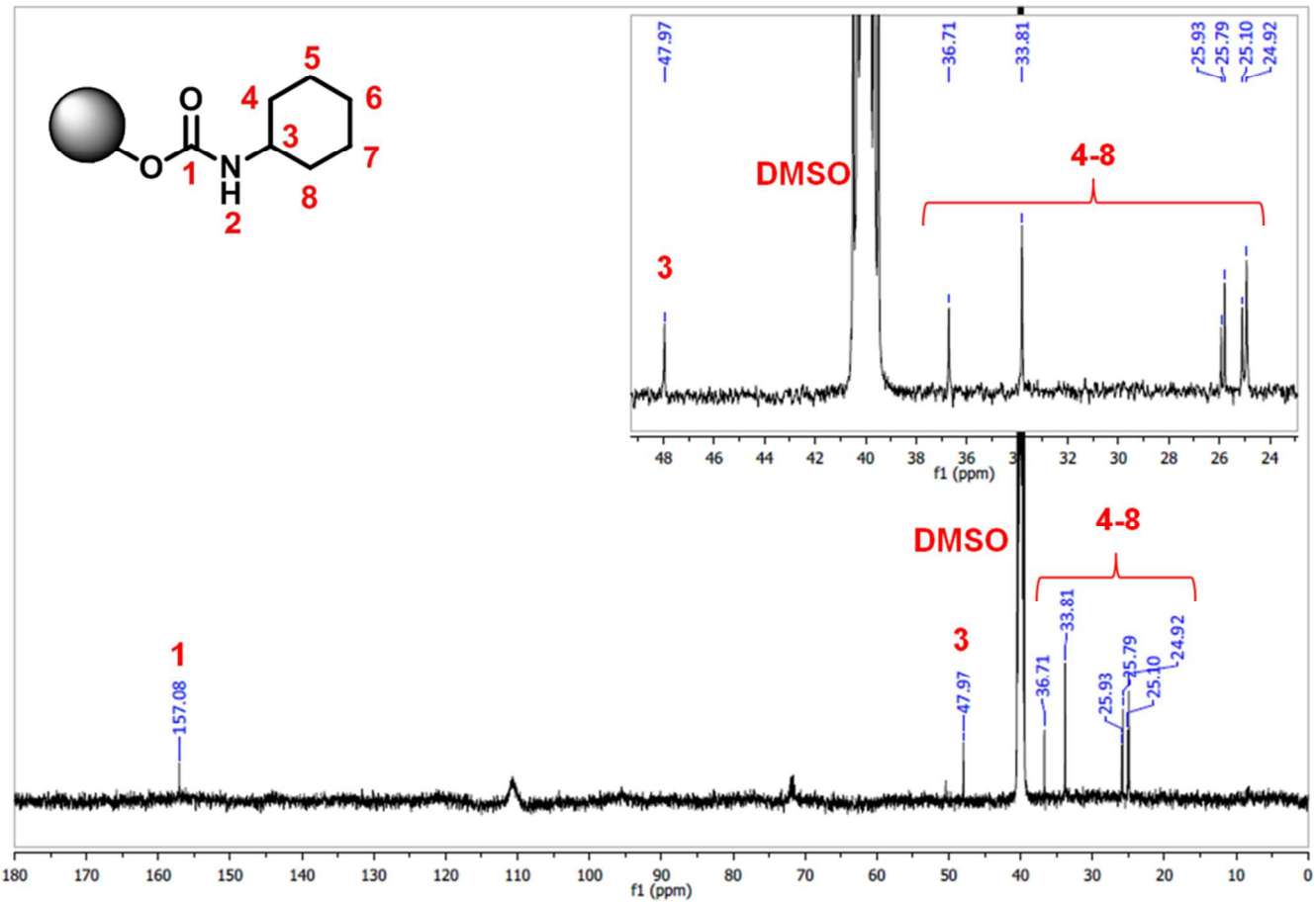
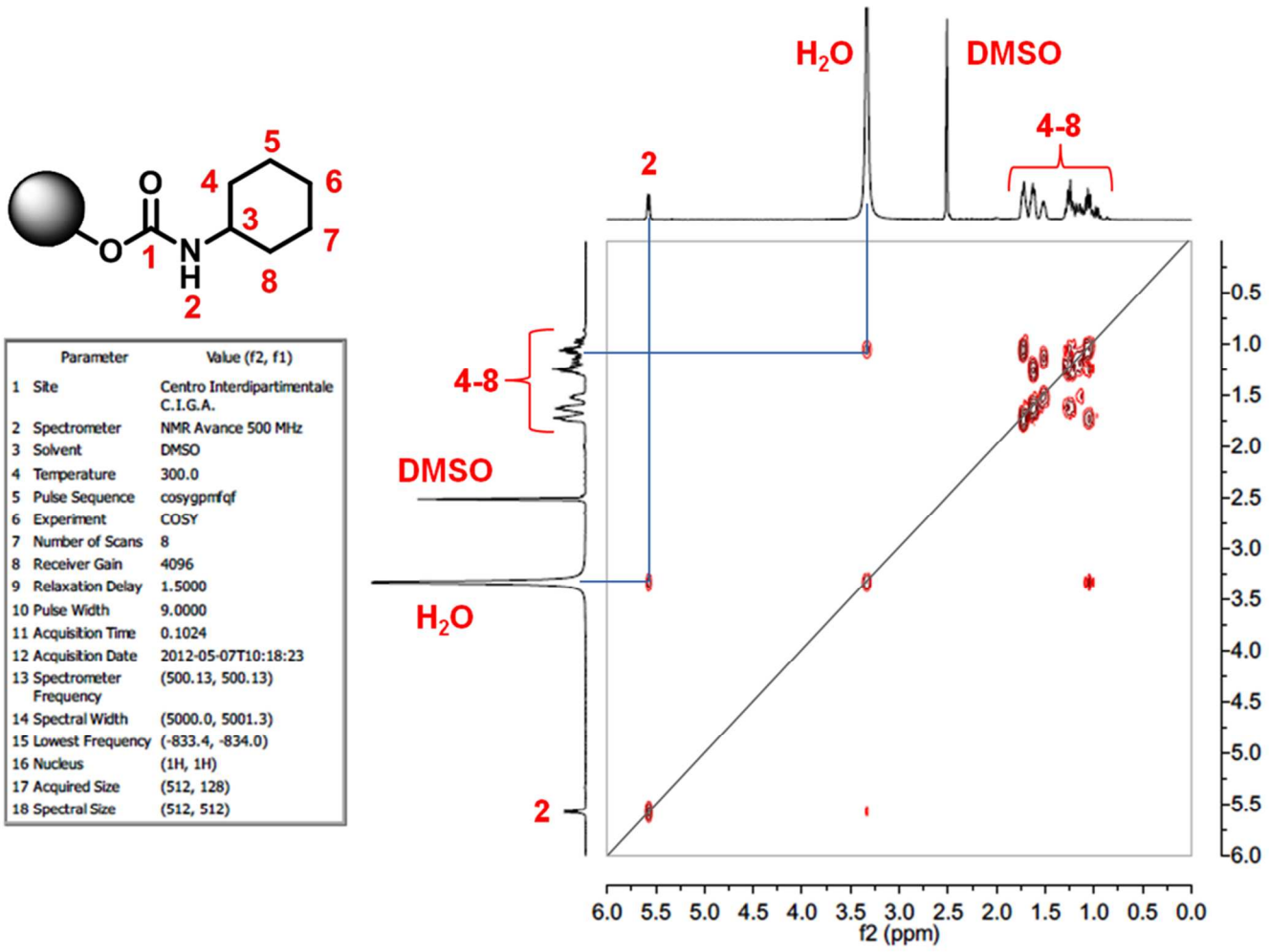
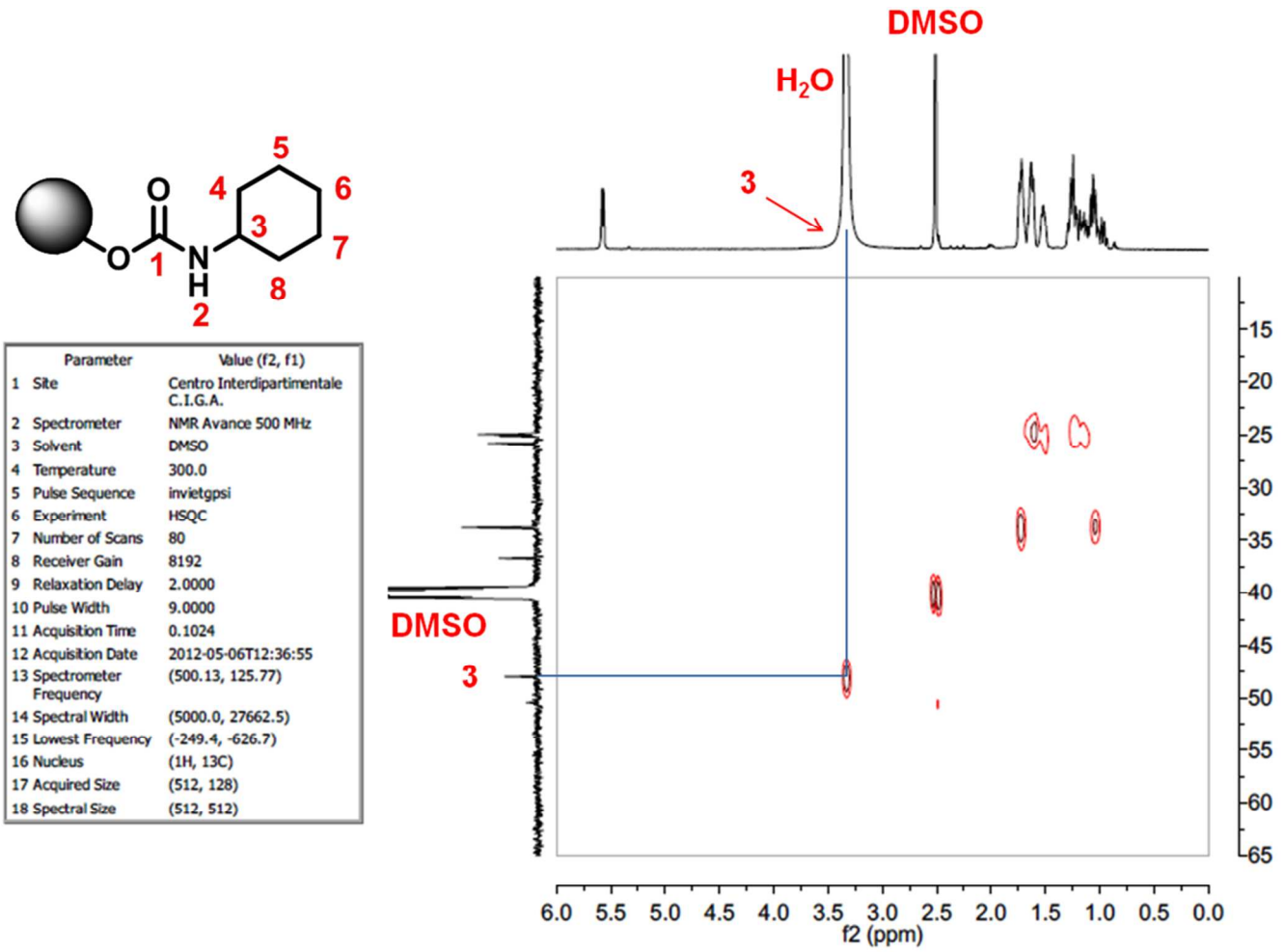


Figure S16 *- ^{13}C all decoupled HR-MAS NMR spectrum of nanoconjugate* **1** *in DMSO (4 KHz speed).*



	Parameter	Value (f2, f1)
1	Site	Centro Interdipartimentale C.I.G.A.
2	Spectrometer	NMR Avance 500 MHz
3	Solvent	DMSO
4	Temperature	300.0
5	Pulse Sequence	cosygpmfqf
6	Experiment	COSY
7	Number of Scans	8
8	Receiver Gain	4096
9	Relaxation Delay	1.5000
10	Pulse Width	9.0000
11	Acquisition Time	0.1024
12	Acquisition Date	2012-05-07T10:18:23
13	Spectrometer Frequency	(500.13, 500.13)
14	Spectral Width	(5000.0, 5001.3)
15	Lowest Frequency	(-833.4, -834.0)
16	Nucleus	(1H, 1H)
17	Acquired Size	(512, 128)
18	Spectral Size	(512, 512)

Figure S17 *- COSY HR-MAS NMR spectrum of nanoconjugate* **1** *in DMSO (4 KHz speed).*



	Parameter	Value (f2, f1)
1	Site	Centro Interdipartimentale C.I.G.A.
2	Spectrometer	NMR Avance 500 MHz
3	Solvent	DMSO
4	Temperature	300.0
5	Pulse Sequence	invietgpsi
6	Experiment	HSQC
7	Number of Scans	80
8	Receiver Gain	8192
9	Relaxation Delay	2.0000
10	Pulse Width	9.0000
11	Acquisition Time	0.1024
12	Acquisition Date	2012-05-06T12:36:55
13	Spectrometer Frequency	(500.13, 125.77)
14	Spectral Width	(5000.0, 27662.5)
15	Lowest Frequency	(-249.4, -626.7)
16	Nucleus	(1H, 13C)
17	Acquired Size	(512, 128)
18	Spectral Size	(512, 512)

Figure S18 *- HSQC HR-MAS NMR spectrum of nanoconjugate* **1** *in DMSO (4 KHz speed).*

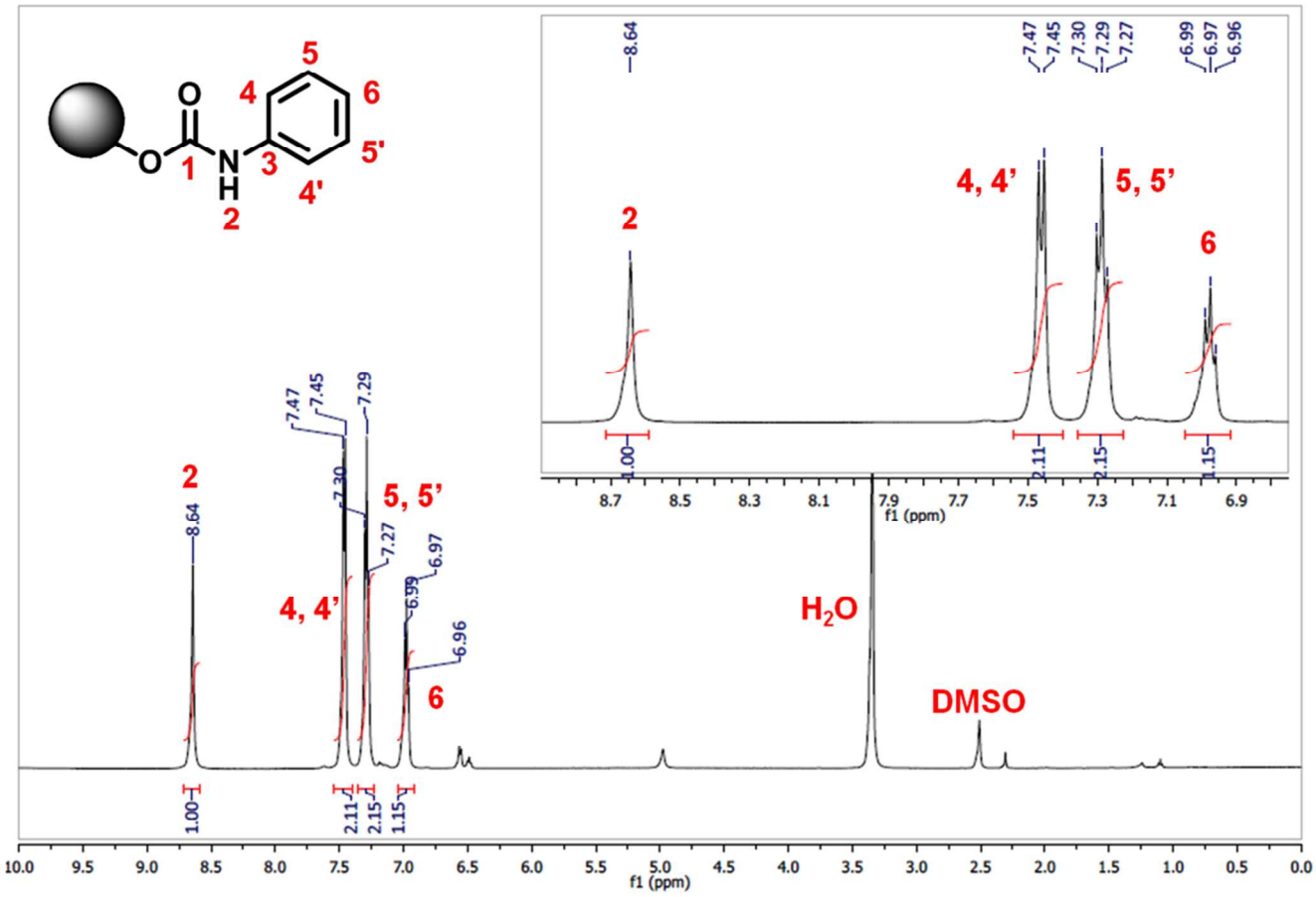


Figure S19 *- ^{1}H HR-MAS NMR spectrum of nanoconjugate* **2** *in DMSO (6 KHz speed).*

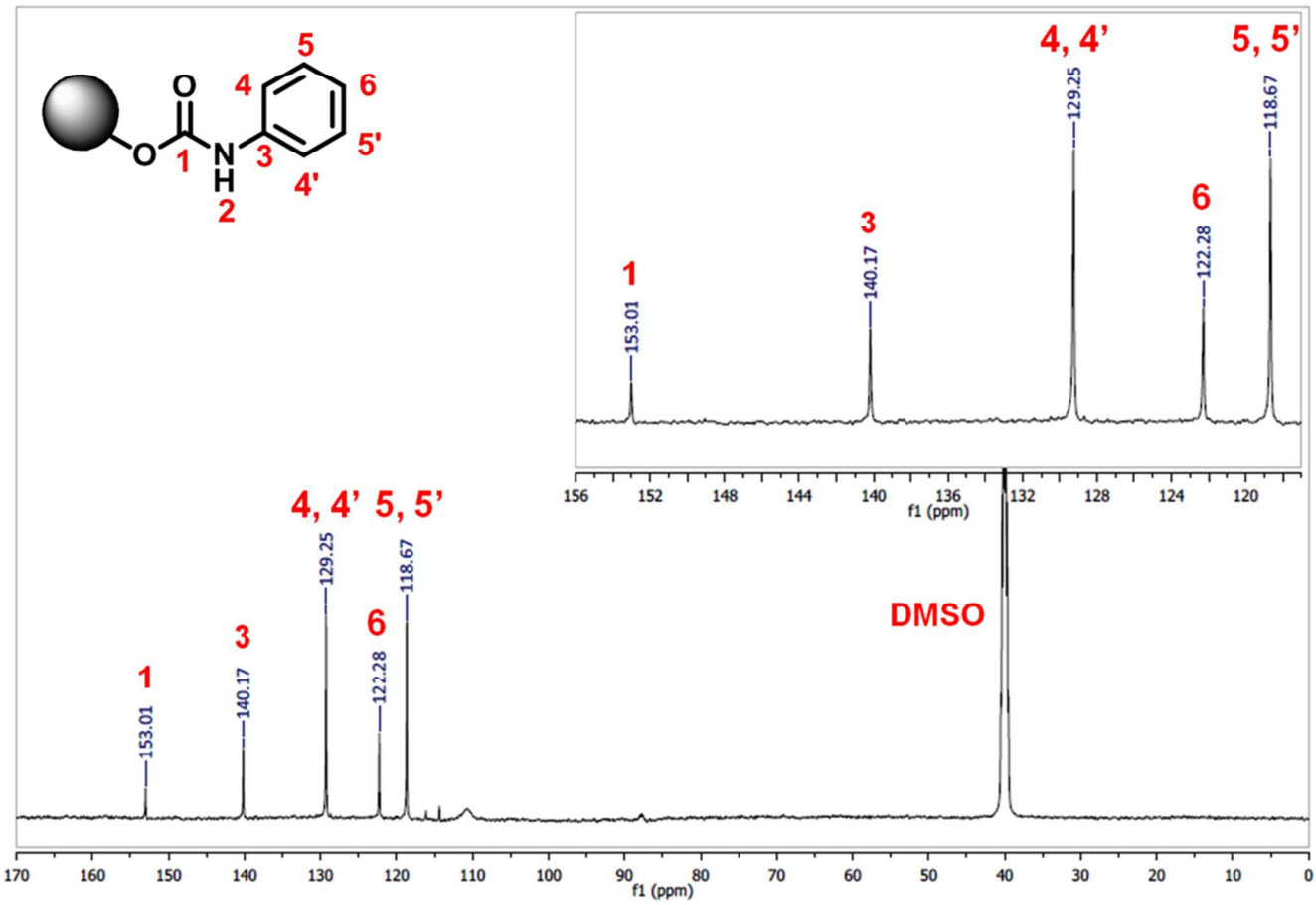


Figure S20 *- ^{13}C all decoupled HR-MAS NMR spectrum of nanoconjugate* **2** *in DMSO (6 KHz speed).*

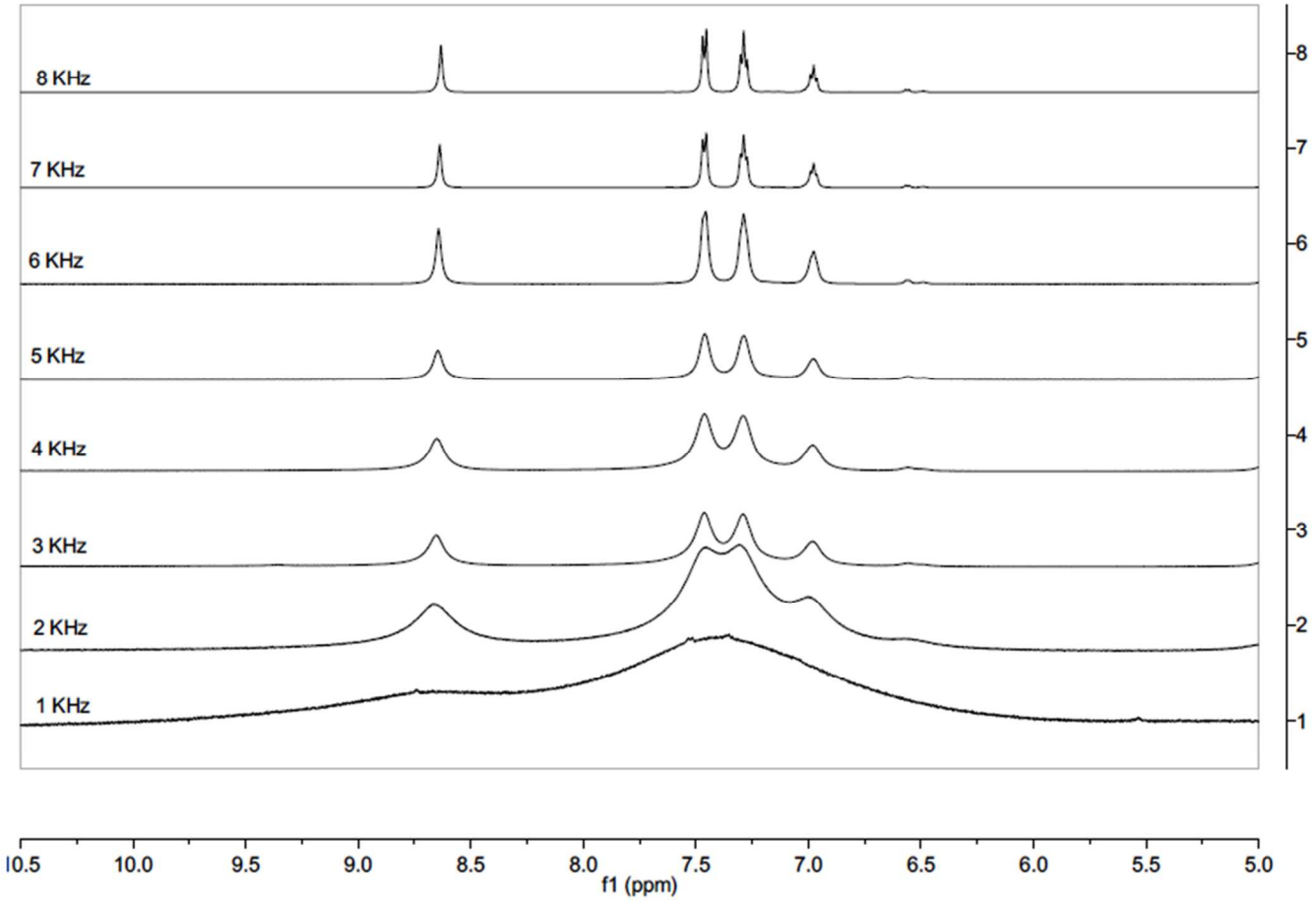


Figure S21 *- Array plot of expanded region of nanoconjugate* **2** *^{1}H spectra acquired at different MAS speed rotations.*

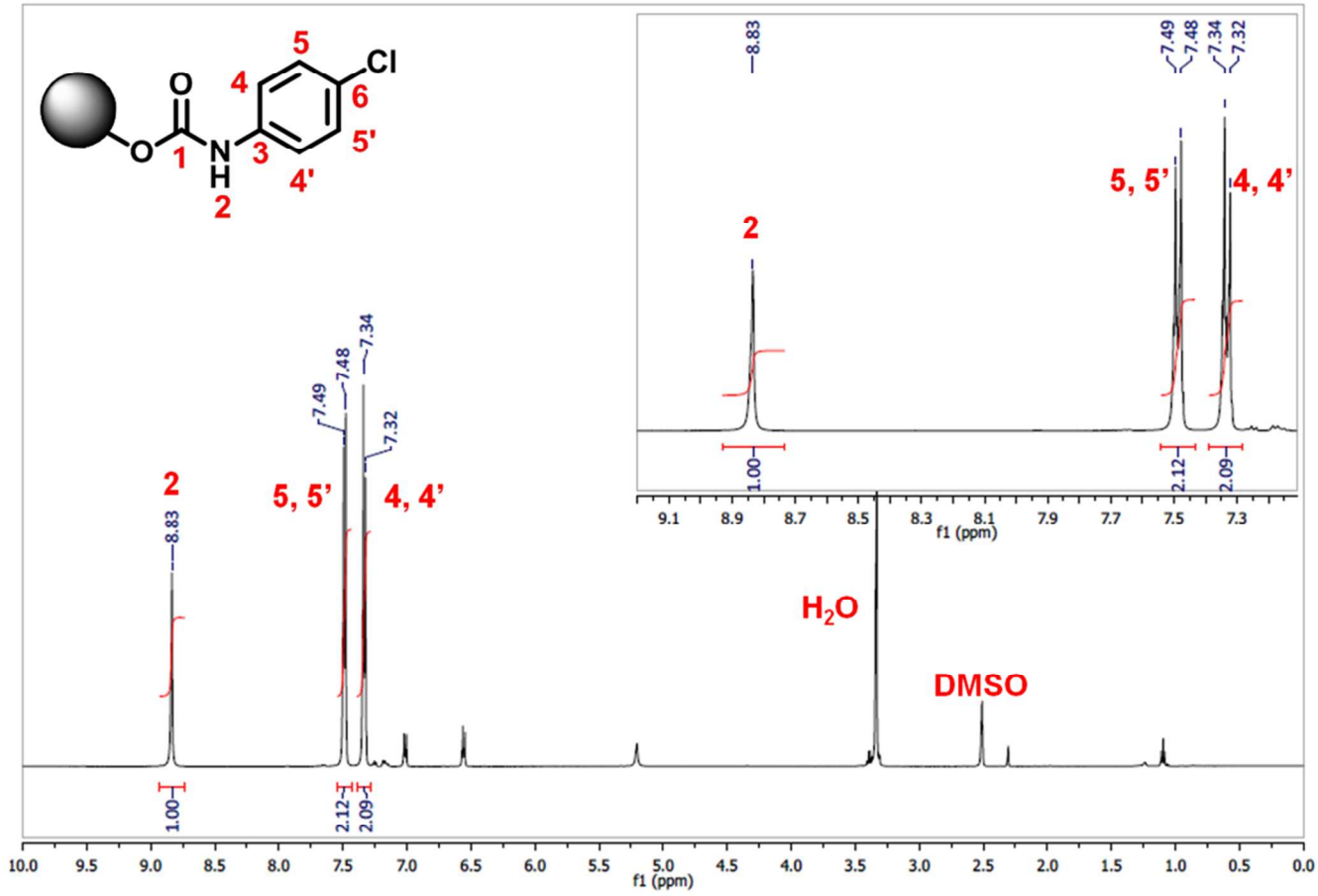


Figure S22 - *^{1}H HR-MAS NMR spectrum of nanoconjugate* **3** *in DMSO (6 KHz speed).*

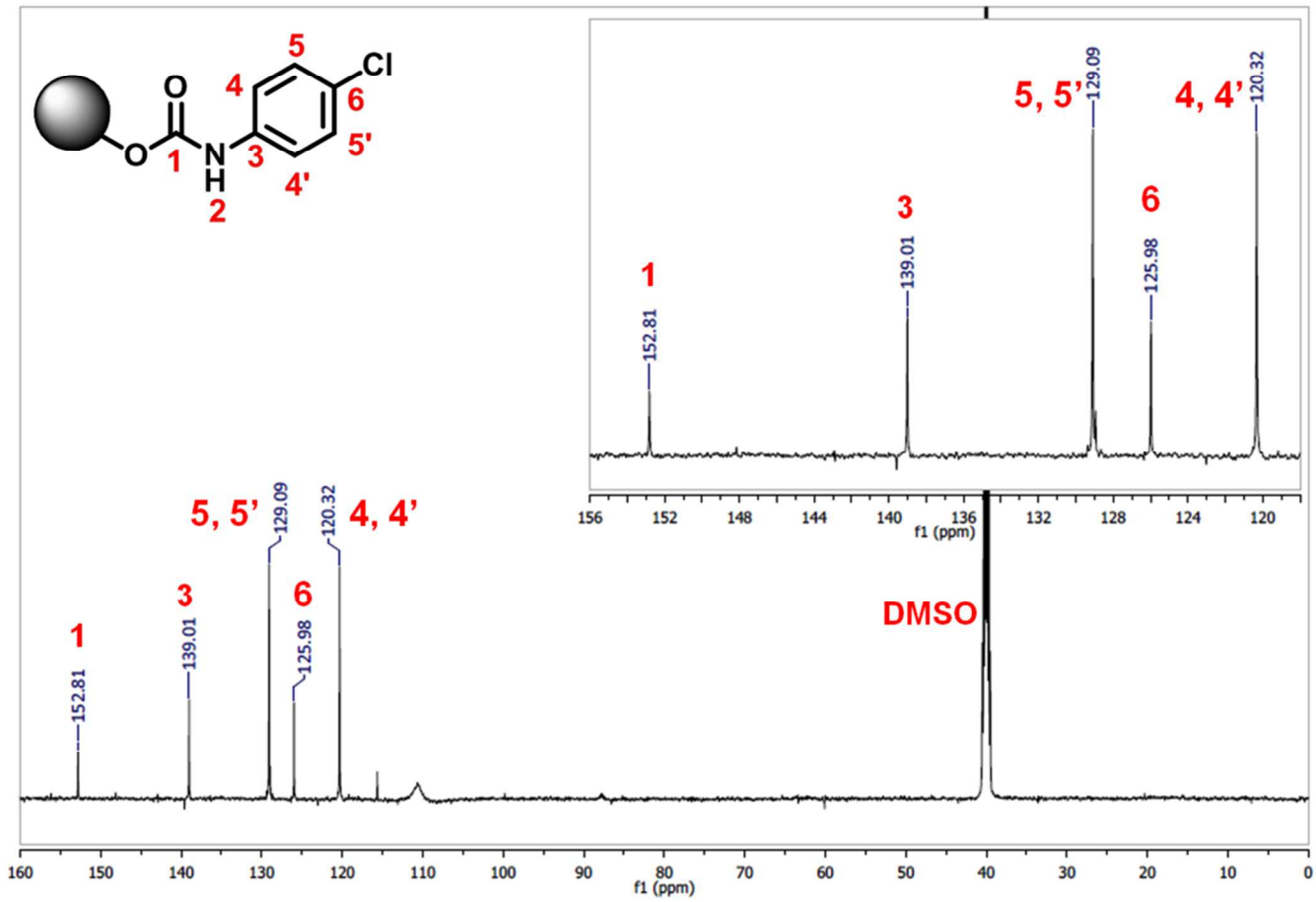


Figure S23 *- ^{13}C all decoupled HR-MAS NMR spectrum of nanoconjugate* **3** *in DMSO (6 KHz speed).*

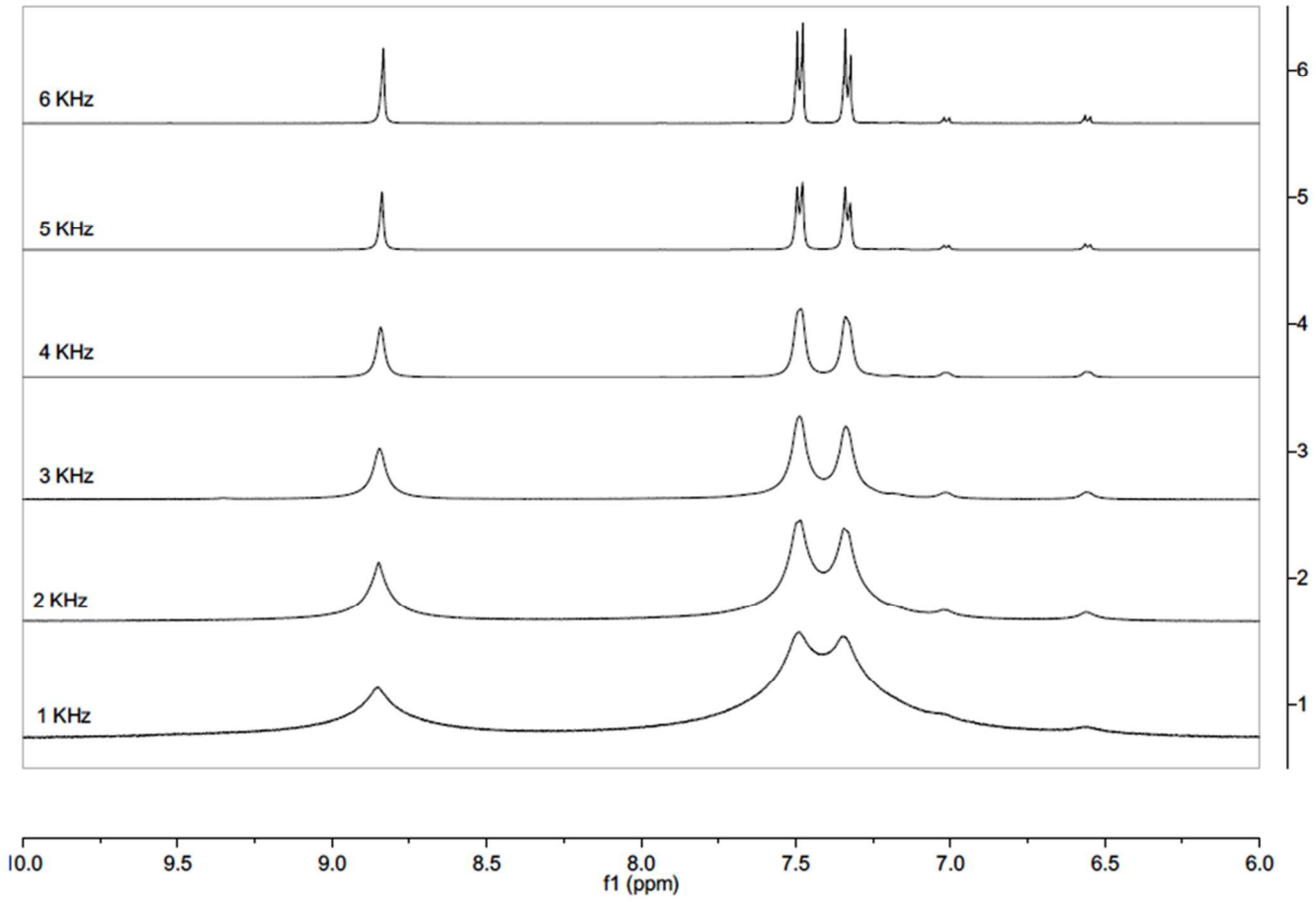


Figure S24 *- Array plot of expanded region of nanoconjugate* **3** *^{1}H spectra acquired at different MAS speed rotations.*

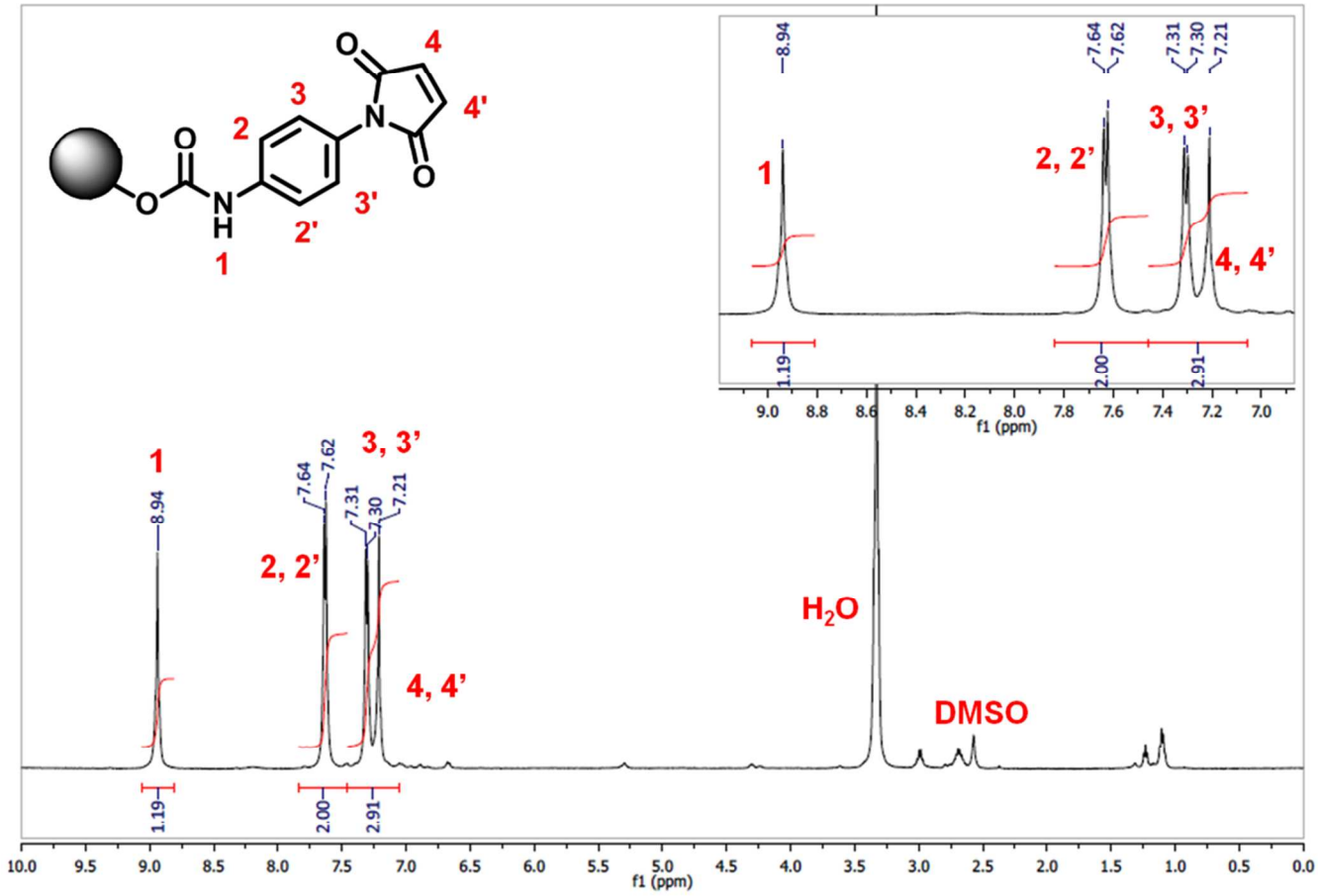


Figure S25 *- ^{1}H HR-MAS NMR spectrum of nanoconjugate* **6** *in DMSO (12 KHz speed).*

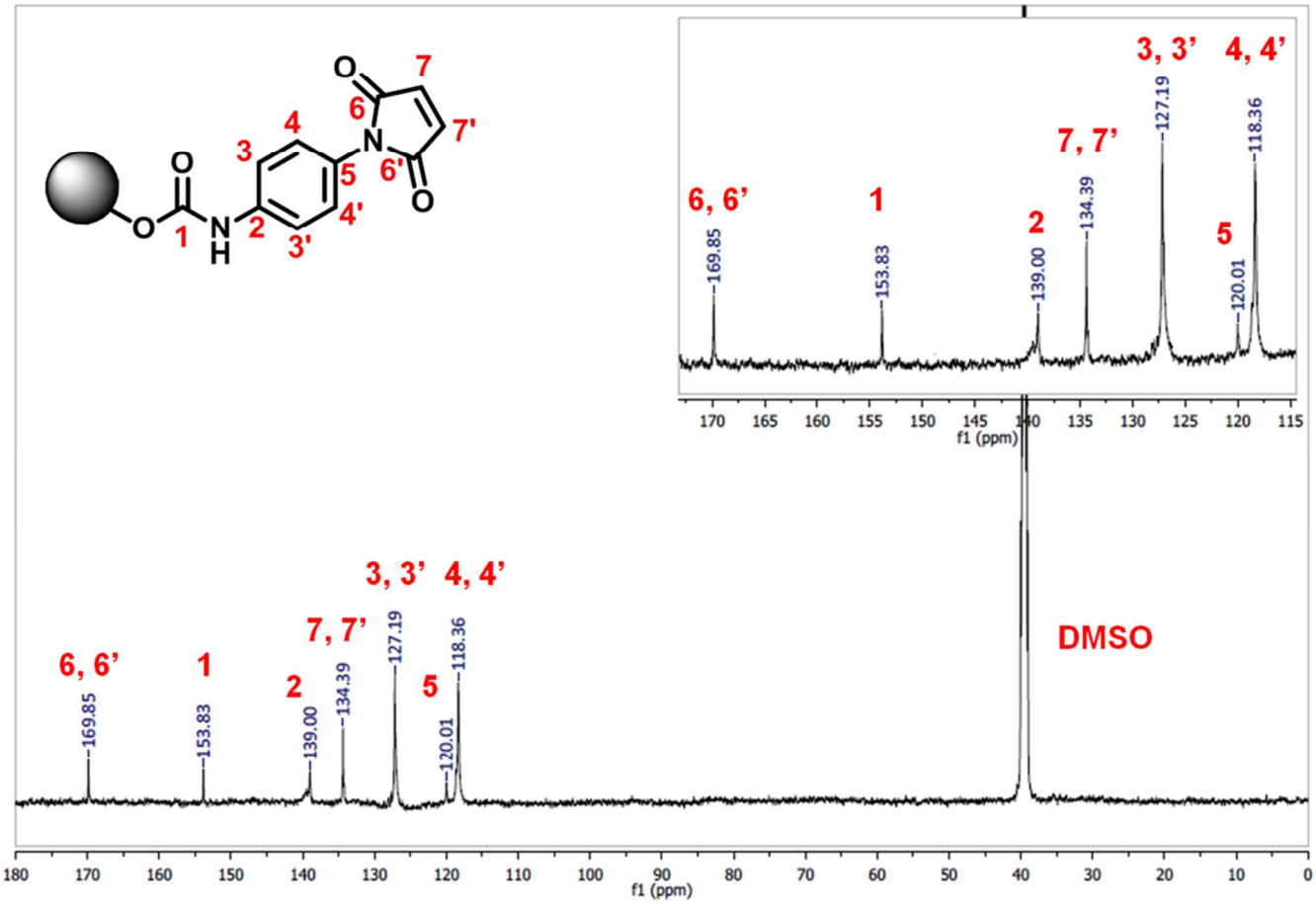


Figure S26 *- ^{13}C all decoupled HR-MAS NMR spectrum of nanoconjugate* **6** *in DMSO (14 KHz speed).*

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[†] Fax: (+39) 02503 14139, Tel: (+39) 02503 14143.

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