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Effect of cyclodextrin complexation on aqueous solubility and photostability of phenothiazine

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The effect of cyclodextrin (β-CD, γ-CD and substituted β-CD derivatives) complexation on the solubility and photostability of phenothiazine (Ph) was compared. The phase solubility method was applied to calculate the stability constants of soluble 1:1 or 1:2 inclusion compounds formed between Ph and CDs. Photochemical decomposition in solution of phenothiazine alone and in the presence of β-CD or β-CD derivatives, was found to proceed according to the two stage first-order reaction and in the case of γ-CD, in a single stage reaction. Formation of solid inclusion complexes of Ph with CDs was evaluated using IR, ^{13}C NMR and DSC studies. The influence of the complexation technique in the solid state (kneading, heating and freeze-drying) on the solubility of Ph was compared. It was establish that the improvement in solubility and stability of Ph was dependent on the kind of CD. When the complexation proceeded in solution it was more effective.

1. Introduction

Phenothiazines, apart from having a transquilizing effect, can show other interesting properties. Moricizine is a phenothiazine derivative with antiarrhythmic properties [1, 2], chlorpromazine and promethazine at relatively high concentrations inhibit lipid peroxidation in rat liver microsoms [3] and phenothiazine dyes are suitable for photodynamic virus inactivation of blood products [4].

Inclusion complexation of phenothiazines with cyclodextrins can solve some problems caused by these drugs, which are: low solubility [2, 5], instability, in particular susceptibility to oxidation [6, 7], as well as hemolysis induced by chlorpromazine [8].

Cyclodextrins are able to form inclusion compounds with a wide variety of molecules, the major requirement being that the guest molecule fits, at least partly, into the cyclodextrin cavity [9]. The effect of cyclodextrin complexation varies depending upon the species of cyclodextrin and drug moiety employed [10–12].

However, some authors have made attempts to induce inclusion of phenothiazines to cyclodextrin but the investigations were performed only for β-CD [8, 13, 14].

The purpose of this study was to obtain inclusion complexes of Ph with different CDs in solution and in the solid state and to examine the influence of the kind of CD and the method of preparation on the solubility and photostability of Ph.

Kneading, heating in sealed ampoules and freeze-drying methods were used for preparation of the inclusion compounds of Ph and CDs in the solid state. The kneading method is particularly useful for poorly water-soluble guest molecules [9]. The freeze-drying procedure is applied primarily for the preparation of complexes with easily water soluble CD derivatives [15–18].

2. Investigations, results and discussion

Phase solubility diagrams of phenothiazine (Ph) and different CDs were obtained at pH = 6.3 and 37 °C (Fig. 1). The solubility of Ph considerably increased as a function of M-β-$CD_{1.8}$ concentration. The increase was slower with an increase of HP-β-$CD_{0.45}$ or β-CD concentration and insignificant with γ-CD. According to the phase diagram classification introduced by Higuchi and Connors [19], the solubility diagrams of Ph and β-CD or β-CD derivatives may be regarded as an A_P type, which means that soluble complexes to a higher order than one in L (CD) were produced (for example SL_2). In turn, for the higher concentrations of γ-CD an A_N type phase diagram was obtained. The origin of the A_N type diagrams was uncertain. It may be associated with an alteration in the effective nature of the solvent in the presence of large concentrations of L (CD) or self-association of L at high concentrations.

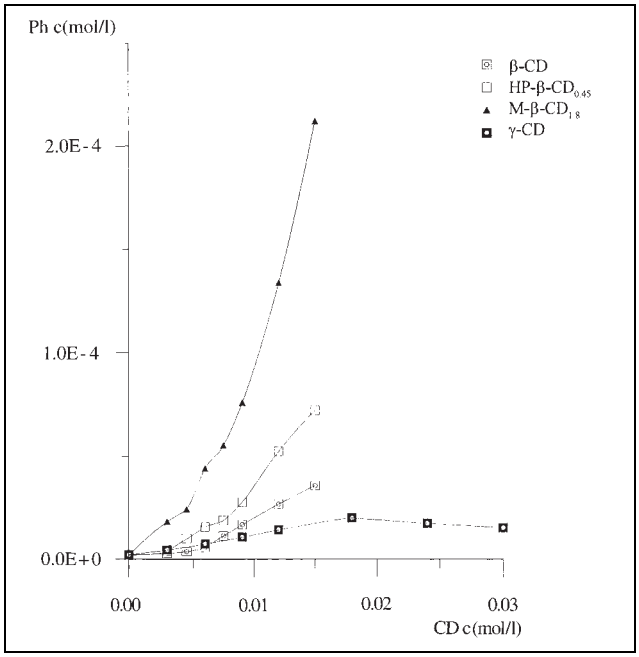


Fig. 1: Phase solubility diagrams of phenothiazine (Ph) and cyclodextrins

The stability constant $K_{1:1}$ of the soluble complex of Ph/γ-CD (1:1) was calculated from the linear section of the solubility diagram as:

$$K_{1:1} = \frac{S}{(1 - S)\, S_0} \qquad (1)$$

where S equals slope and S_0 equals solubility of Ph in the absence of CD.

For 1:1 and 1:2 complexes of Ph and β-CD or β-CD derivatives, the plots of $(S_t - S_0)/L_t$ against L_t yielded a straight line, thus from the slope and the intercept, values for $K_{1:1}$ and $K_{1:2}$, were estimated according to eq. (2):

$$\frac{S_t - S_0}{L_t} = K_{1:1}S_0 + K_{1:1}K_{1:2}S_0L_t \qquad (2)$$

Table 1: Stability constants ($K_{1:1}$, $K_{1:2}$) of inclusion complexes of phenothiazine with cyclodextrins

Cyclodextrin	$K_{1:1}$ (mol^{-1})	$K_{1:2}$ (mol^{-1})
β-CD	140	600
M-β-$CD_{1.8}$	1090	339
HP-β-$CD_{0.45}$	55	2884
γ-CD	532	–

where S_0 is equlibrium solubility of Ph in the absence of CD, S_t is total concentration of dissolved Ph and L_t is total concentration of added CD.

Determined, in this way, the apparent $K_{1:1}$ and $K_{1:2}$ values of inclusion compounds of Ph with different CDs are compiled in Table 1.

The influence of CDs on the changes of Ph concentration during exposure to UV polychromatic (200–400 nm) radiation was followed using UV spectrophotometry ($\lambda_{max} = 254$ nm, spectra scaned from 200 to 400 nm).

The photodecomposition of Ph in ethanolic solution (too low water solubility) and in complexes with CDs in aqueous solution obeyed first-order kinetics. The shape of phenothiazine decomposition curves presented in Fig. 2 suggests that the photodecomposition may proceed by coupled sequence reactions in the case of Ph alone and its complexes with β-CD or β-CD derivatives and by a single stage reaction for the Ph/γ-CD complex. The first-order rate constants of the first and second stage of photodecomposition reaction of phenothiazine alone and phenothiazine in CDs complexes indicate that β-CD and its methylated or hydroxypropylated derivatives did not significantly affect the rate of the phenothiazine decomposition reaction; γ-CD however, improved the photostability of phenothiazine (Table 2).

This phenomenon can be explained by taking into consideration the apparent stability constants of the Ph/CD complexes, calculated from the phase solubility diagrams (Table 1, Fig. 1). It follows, that the γ-CD forms with phenothiazine an 1 : 1 complex. However, the large inside cavity of γ-CD makes possible more precise inclusion of the whole moiety of phenothiazine, which results in a better protective effect. β-CD and its derivatives, which have a smaller inside cavity, formed with Ph the mixture of 1 : 1 and 1 : 2 complexes. In the 1 : 1 complex, a part of the phenothiazine moiety remained outside the cavity. In this case the cyclodextrin probably could not function in a protective role.

Table 2: Kinetic parameters for photochemical decomposition of phenothiazine (Ph) in inclusion complexes with cyclodextrins in solution

Compound	k_1[a] (s^{-1})	$t_{0.5}$[b] (min)	$t_{0.1}$[c] (min)	k_2[a] (s^{-1})
Ph in et sol.	9.53×10^{-4}	12	2	4.64×10^{-6}
Ph + β-CD in aq. sol.	3.65×10^{-4}	32	5	5.60×10^{-6}
Ph + M-β-$CD_{1.8}$ in aq. sol.	8.15×10^{-4}	14	2	1.51×10^{-5}
Ph + M-β-$CD_{0.97}$ in aq. sol.	2.63×10^{-4}	44	7	9.0×10^{-6}
Ph + HP-β-$CD_{0.45}$ in aq. sol.	4.15×10^{-4}	28	4	3.18×10^{-6}
PH + γ-CD in aq. sol.	5.67×10^{-6}	2038	310	–

[a] k_1, k_2: I-order reaction rate constants
[b] $t_{0.5}$: half-degradation time
[c] $t_{0.1}$: time of 10% decomposition

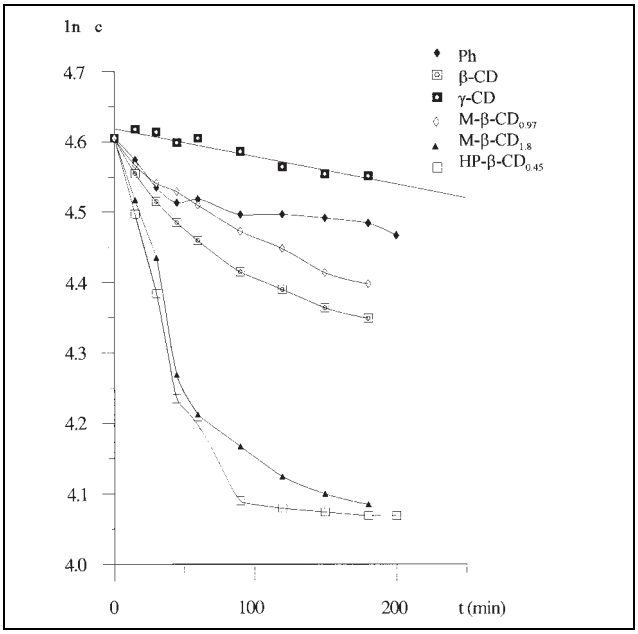


Fig. 2: Changes of phenothiazine concentration during exposition to UV radiation the solutions of: phenothiazine (Ph) and phenothiazine complexes with β-CD, γ-CD, M-β-$CD_{0.97}$, M-β-$CD_{1.8}$ and HP-β-$CD_{0.45}$

The inclusion complexes of Ph with CD in the solid state were obtained using "kneading", "heating" and "freeze-drying" methods. The solubilities of physical mixtures and adequate products of these three complexation methods are compared in Table 3 and indicate that the greatest improvement yielded kneading or heating of Ph with M-β-$CD_{1.8}$ or HP-β-$CD_{0.45}$. Freeze-drying, however, appears to be an unfavourable method for Ph/CDs complexation. The unsuccessful results of freeze-drying can be explained on the A_P type solubility diagrams in the case of all the investigated CDs except for γ-CD. According to Funk et al. [16], if at the eutectic point the solubility of the inclusion compound is not exceeded, no solid inclusion compound precipitates during the freeze drying process. On the contrary, the phase solubility diagram of Ph/γ-CD was classified as A_N type, which means that above the defined γ-CD concentration the Ph-solubility decreased and hence, probably, the freeze-drying process resulted in Ph/γ-CD complex precipitates. This is why, in this one case, the solubility of the freeze-drying product was high relative to that of precipitates with other CDs.

In order to confirm the inclusion of Ph into the CDs cavity, the structure of solid samples was evaluated using IR and ^{13}C NMR spectroscopies and differential scanning calorimetry.

Table 3: Comparison of water solubility of phenothiazine (Ph) in physical mixtures with cyclodextrins and in adequate samples kneaded, heated and freeze-dried

Cyclodextrin in mixture with Ph	PM ($\mu g/cm^3$)	K ($\mu g/cm^3$)	H ($\mu g/cm^3$)	F-D ($\mu g/cm^3$)
β-CD	0.44	0.50	0.75	0.04
γ-CD	0.50	0.47	0.37	0.33
M-β-$CD_{1.8}$	0.74	1.33	1.22	–
DM-β-CD	0.39	–	–	0.12
TM-β-CD	0.06	–	–	0.16
HP-β-$CD_{0.45}$	0.32	1.21	0.75	–
HP-β-$CD_{0.6}$	0.94	–	–	0.25
Ph		0.40		

PM: Physical mixture, K: sample kneaded, H: sample heated, F-D: sample freeze-dried

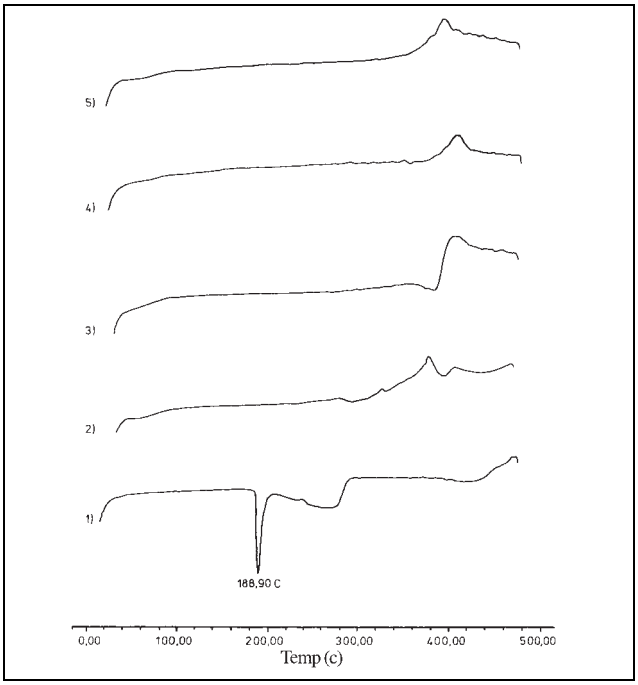


Fig. 3: DSC curves: Ph(1), M-β-$CD_{1.8}$ (2) and Ph/M-β-$CD_{1.8}$ physical mixture (3), sample kneaded (4), sample heated (5)

A comparison of IR spectra of physical mixtures and samples kneaded, heated or freeze-dried with β-CD derivatives did not reveal any changes. However, the IR spectra of Ph/γ-CD kneaded and freeze dried products demonstrated a shortening of the N−H bending band at 1570 cm^{-1} and the disappearance of the 735 cm^{-1} band, which is assignable to the out-of plane bending vibrations of the 4 adjacent hydrogens of the phenothiazine ring system. This disappearance can be also seen in the IR spectra of Ph/M-β-$CD_{1.8}$ products, where additionally the intensity of the bending CH_2 band at 1440 cm^{-1} decreased.

The characteristic endotherm on the DSC thermogram of Ph at 188.9 °C corresponds to its m.p. (Figs. 3–5). The thermograms of Ph/M-β-$CD_{1.8}$ products kneaded and heated did not differ from the physical mixture, since the Ph endotherm was also not present on the physical mixture curve (Fig. 3). In turn, all DSC thermograms of Ph/HP-β-$CD_{0.45}$ samples showed the presence of a small amount of unbounded Ph (Fig. 4). In the case of samples with γ-CD, the Ph endotherm was not marked out on the DSC curves of the kneaded and freeze-dried products (Fig. 5).

Table 4 summarizes the effects of CDs on the ^{13}C-chemical shifts of Ph. The carbon signals of Ph were only insignificantly affected by the presence of CDs. The shieldings of C in Ph/M-β-$CD_{1.8}$ samples indicated no complex formation during kneading. The shieldings of C1, C8 and C3, C6 in the Ph/HP-β-$CD_{0.45}$ kneading product cannot confirm the complex formation, because the same shifts appeared in the spectrum of the physical mixture of these components. The shifts of C1-C8 of Ph kneaded or freeze-dried with γ-CD could indicate a hydrophobic interaction of host/guest-molecules as well as the changes in its structure.

The results of these investigations demonstrate that, although, the aqueous solubility of Ph in the presence of β-CD derivatives increased, no inclusion compound existed in the kneaded or heated products, but a physical mixture of these components was produced. This solubil-

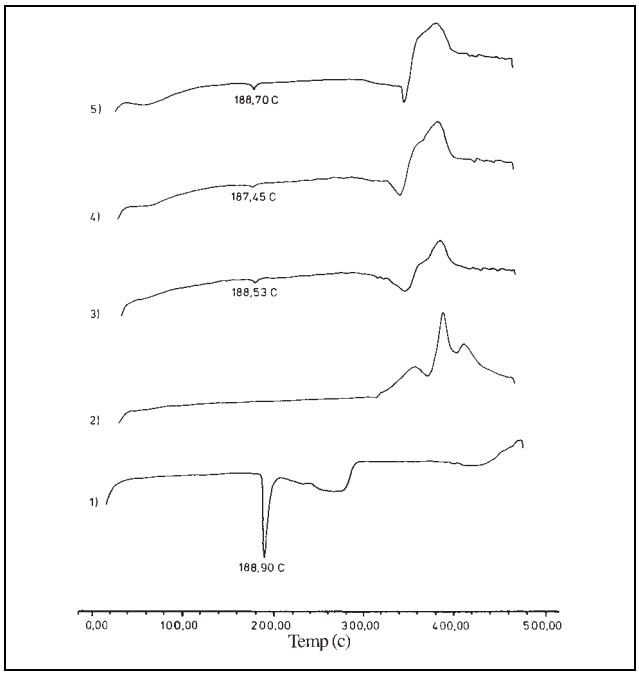
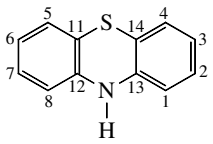


Fig. 4: DSC curves: Ph (1), HP-β-$CD_{0.45}$ (2) and Ph/HP-β-$CD_{0.45}$ physical mixture (3), sample kneaded (4), sample heated (5)

Table 4: Values of chemical shifts of phenothiazine (Ph) and its complexes with cyclodextrins



C	Ph (ppm)	Chemical shifts Δδ[a]						
		Ph/M-β-$CD_{1.8}$		Ph/HP-β-$CD_{0.45}$		Ph/γ-CD		
		PM[b]	K	PM	K	PM	K	F-D
12.13	142.102	+0.026	+0.023	+0.049	+0.049	+0.068	+0.076	+0.087
1.8	127.521	+0.050	+0.042	+0.103	+0.103	+0.129	+0.141	+0.148
2.7	126.231	+0.035	+0.027	+0.084	+0.084	+0.110	+0.118	+0.129
3.6	121.751	+0.045	+0.038	+0.106	+0.106	+0.144	+0.151	+0.167
11.14	116.340	0	−0.003	+0.038	+0.038	+0.061	+0.072	+0.088
4.5	114.413	+0.034	+0.030	+0.076	+0.063	+0.099	+0.106	+0.118

[a] $\Delta\delta = \delta_{PM}$, δ_K or $\delta_{F-D} - \delta_{Ph}$
[b] PM: physical mixture, K: sample kneaded, F-D: sample freeze-dried

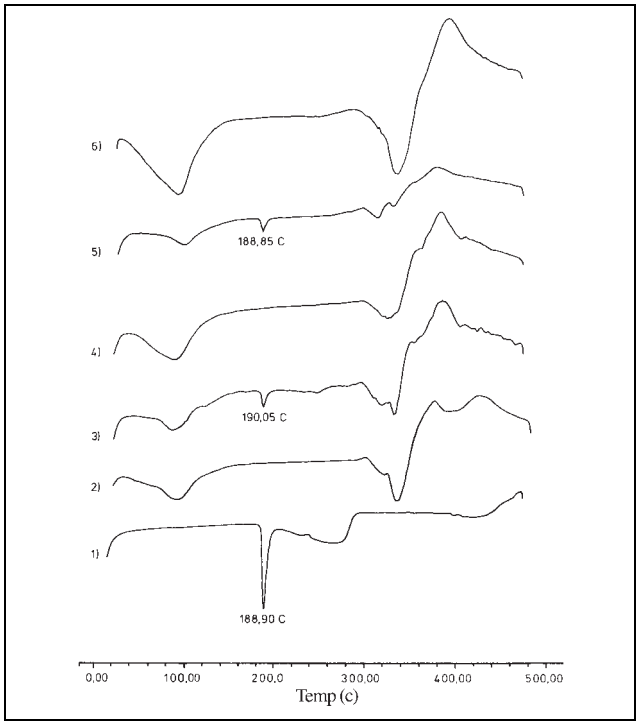


Fig. 5: DSC curves: Ph (1), γ-CD (2) and Ph/γ-CD physical mixture (3), sample kneaded (4), sample heated (5), sample freeze-dried (6)

ity improvement can be explained by greater specific areas or the amorphous state of the samples, as a result of their preparation. In contrast, the results obtained for γ-CD indicate complex formation, but the complex solubility is limited by the lower aqueous solubility of γ-CD, in comparison to the β-CD derivatives.

In conclusion it can be said, that the complexation methods in solution appear to be more advantageous for obtaining Ph/CD inclusion compounds than the solid state.

3. Experimental

3.1. Materials

Phenothiazine (Sigma 73H3418) and cyclodextrins were used for the studies: β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), methyl-β-cyclodextrin MS = 0.97 (M-β-$CD_{0.97}$), methyl-β-cyclodextrin MS = 1.8 (M-β-$CD_{1.8}$), hydroxypropyl-β-cyclodextrin MS = 0.45 (HP-β-$CD_{0.45}$) (these CDs were gifts from Prof. Dr. B. W. Müller – Department of Pharmaceutics and Biopharmaceutics, University of Kiel, Germany), hydroxypropyl-β-cyclodextrin MS = 0.6 (HP-β-$CD_{0.6}$) (Aldrich 15915DQ), heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) (Sigma 56H0347), heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) (Sigma 76H0503).

3.2. Phase solubility studies (acc. [19])

Increasing amounts of suitable cyclodextrin solution (from 3×10^{-3} to 15×10^{-3} mol/l^{-1} or to 30×10^{-3} in the case of γ-CD) were added to eight 10 ml ampoules containing 10 mg of phenothiazine and the suspensions were adjusted with water to a volume of 5 cm^3. The ampoules were shaken for 30 h in the dark in a water bath at 310 K and then stored for 100 h at 277 K. The suspensions were filtered (membrane filter 0.45 μm) and the concentration of Ph was determined by UV spectroscopy (spectrophotometer UV-160A Shimadzu) at λ = 254 nm. The stability constants $K_{1:1}$ and $K_{1:2}$ of the complexes obtained were calculated.

3.3. Kinetic studies

Phenothiazine in ethanolic solution or inclusion complexes of phenothiazine with cyclodextrins in aqueous solution were exposed to UV polychromatic radiation (200–400 nm, distance 20 cm) in quartz cuvette cells (1 cm). The changes of phenothiazine concentration were followed by UV spectroscopy at λ = 254 nm. To determine the kinetic parameters of photodegradation of phenothiazine a kinetic model of the first-order reaction was used. The results were interpreted by the subtraction technique, employing the method of least squares.

3.4. Preparation of solid complexes

3.4.1. Kneading method

Phenothiazine and the selected CD in a 1:1 molar ratio were triturated 30 min in an agate mortar with 0.5 ml ethanol. The paste, thus obtained, was dried under reduced pressure at room temperature for 24 h.

3.4.2. Heating method

10 mg of 1:1 physical mixtures of Ph with CD placed in sealed glass ampoules were stored in a thermostated chamber 36 h at 363 K and then 100 h at 277 K.

3.4.3. Freeze-drying method

Ph (30 mg) was dissolved in 10 ml ethanol and added dropwise to 10 ml CD aqueous solution (1.5×10^{-2} mol/l) and then stirred for 90 min at 310 K. The obtained solution, after ethanol evaporation, was frozen and lyophilized for 30 h (Leybold, Germany). The solid product was stored for 48 h under reduced pressure at room temperature.

3.5. Solubility studies

The solubility studies were performed according to the method described previously [20]. The concentration of Ph in filtrate was determined spectophotometrically at 254 nm (UV-160A spectrophotometer, Shimadzu).

3.6. Infrared absorption spectroscopy (IR)

IR spectra were obtained for KBr discs (1.5 mg sample + 300 mg KBr) using a Specord M-80 spectrophotometer, Carl Zeiss Jena.

3.7. Differential scanning calorimetry (DSC)

The DSC patterns were carried out for the solid samples (2.0–2.4 mg) from 25 °C to 480 °C (298 K–653 K), under a N_2 stream (20 cm^3/min), at a scanning speed of 20 °C/min with a DSC-50 Shimadzu Apparatus.

3.8. ^{13}C NMR spectroscopy

^{13}C NMR spectra were recorded using a Varian Gemini 300VT Fourier Transform spectrometer at 75.46 MHz. Chemical shifts were measured relative to internal solvent DMSO at 39.50 ppm.

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