Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Convenient Detection of Thiol Functional Group Using H/D Isotope Sensitive Raman Spectroscopy

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EXPERIMENTAL DETAILS

Thiophenol (99+%) was purchased from Spectrochem Pvt. Ltd. 1-ethanethiol (99+%) and deutereted water (99.8%) were purchased from Acros Organics. 1-butanethiol (99%), 1-octanethiol (98.5%), 1-dodecanethiol (98+%), cysteamine hydrochloride, cystamine, L-cysteine hydrochloride, L-cystine, N-acetyl cysteine, chloroform-D (99.8+%) and HPLC grade methanol (99.8+%) were purchased from Aldrich chemical company. Glutathione and glutathione disulfide (extrapure) were purchased from SRL Pvt. Ltd. Methanol-D4 (99.8+%) was purchased from Merck Germany. All reagents were used without any further purification. The single distilled water was further distilled twice by using Borosil distillation setup and this triply distilled water was used for sample preparation.

The neat deuterated samples for PhSH and $C_8H_{17}SH$ (as in Fig. 2a & 2b in main text) are prepared by dissolving the crude (liquid) compounds in 10 equivalents of CD_3OD, and then by evaporating the solvent by rotary evaporation. The Raman spectra of the deuterated species of other alkyl thiols (C_2H_5SH, C_4H_9SH and $C_{12}H_{25}SH$) were recorded in solutions of CD_3OD. The spectrum of CD_3OD is subtracted from the solution spectra to get the spectra of the deutereted compounds. Similarly the spectra for the protonated thiols are acquired in CH_3OH. Data in water are obtained by dissolving the compounds in H_2O or D_2O (for isotopic substitution).

A Kr^+ laser (Sabre Innova, model SBRC-DBW-K) from Coherent and a spectrograph with 1.5 mm slit width (model Trivista 555) fitted with an electronically cooled Pixis CCD from Princeton Instruments are used to collect the Raman data. The excitation wavelength used in the Raman experiments is 482.5 nm and the power applied

on the sample is around 30 mW. Spectra of liquid alkylthiols (both neat and solution) are recorded in NMR tubes.

All NMR spectra were recorded on the Bruker DPX-400 spectrometer at room temperature.

All the DFT calculations were performed on the Inorganic-HPC cluster at IACS using the Gaussian 03 software package.[1] The geometries were optimized with the spin-unrestricted formalism using both the BP86 functional and the 6-311G* basis set. Frequency calculations were performed on each optimized structure using the same basis set to ensure that it was a minimum on the potential energy surface. The Raman spectra are calculated using the Raman key word in Gausian 03 package.

^{1}H NMR

PhSH: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.32 (m, 4H), 7.22 (m, 1H), 3.51 (s, 1H).

PhSH: ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm): 7.25-7.06 (m, 5H).

C_2H_5SH: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.54 (m, 2H), 1.37 (t, 1H), 1.31 (t, 3H).

C_2H_5SH: ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm): 2.50 (q, 2H), 1.29 (t, 3H).

$C_8H_{17}SH$: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.50 (q, 2H), 1.60 (m, 2H), 1.40-1.27 (m, 11H), 0.87 (t, 3H).

$C_8H_{17}SH$: ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm): 2.48 (q, 2H), 1.60 (m, 2H), 1.42-1.24 (m, 10H), 0.89 (t, 3H).

Glutathione: ^{1}H NMR (D_2O, 400 MHz) δ (ppm): 4.59 (t, 1H), 4.00 (s, 2H), 3.85 (t, 1H), 2.97 (q, 2H), 2.58 (m, 2H), 2.98 (m, 2H).

S1-S7: ^{1}H NMR spectra of thiols

Fig. S1. PhSH in $CDCl_3$

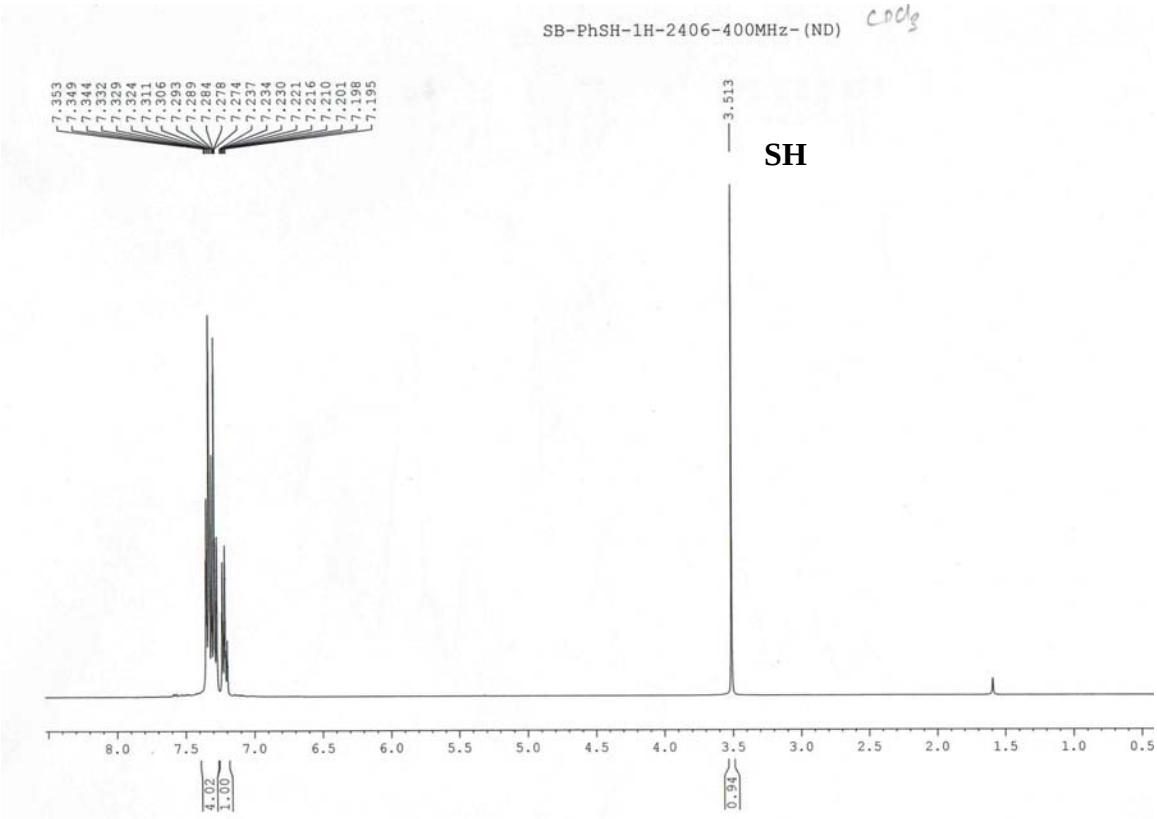

Fig. S2. PhSH in CD_3OD

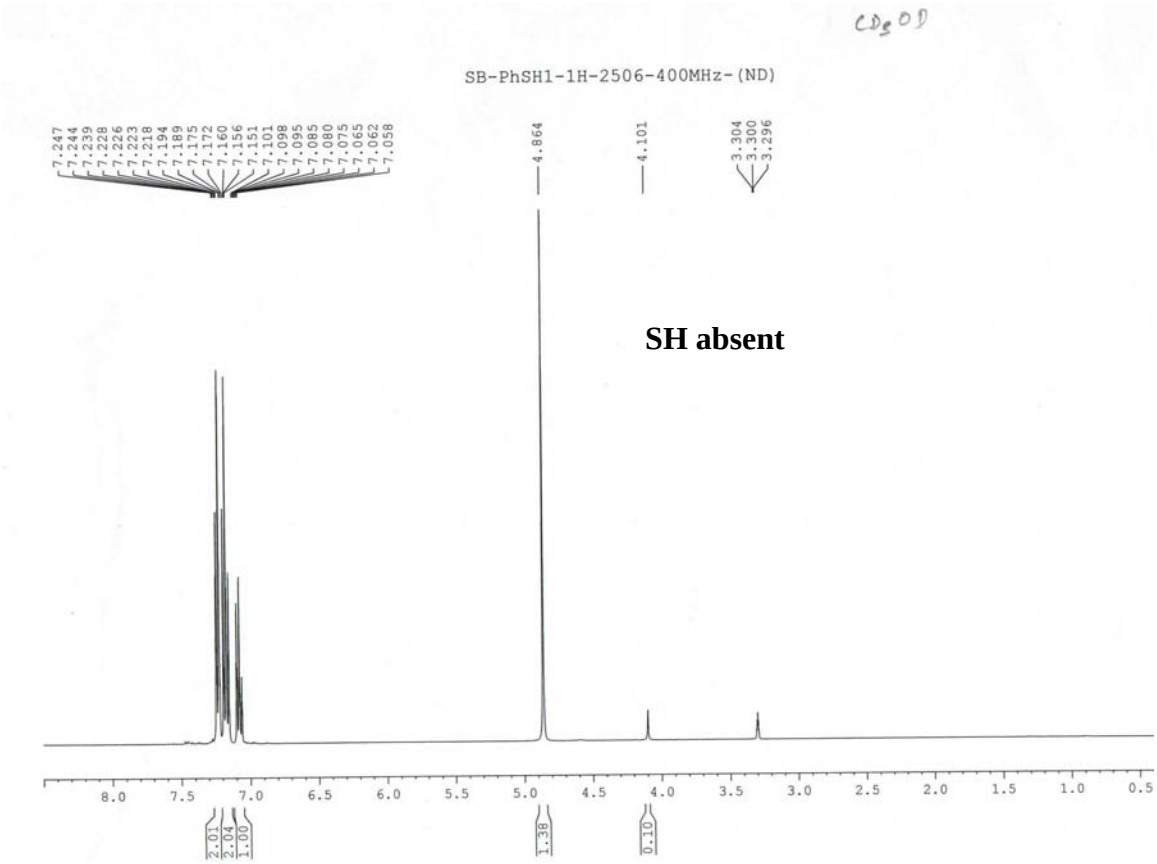

Fig. S3. C_2H_5SH in $CDCl_3$

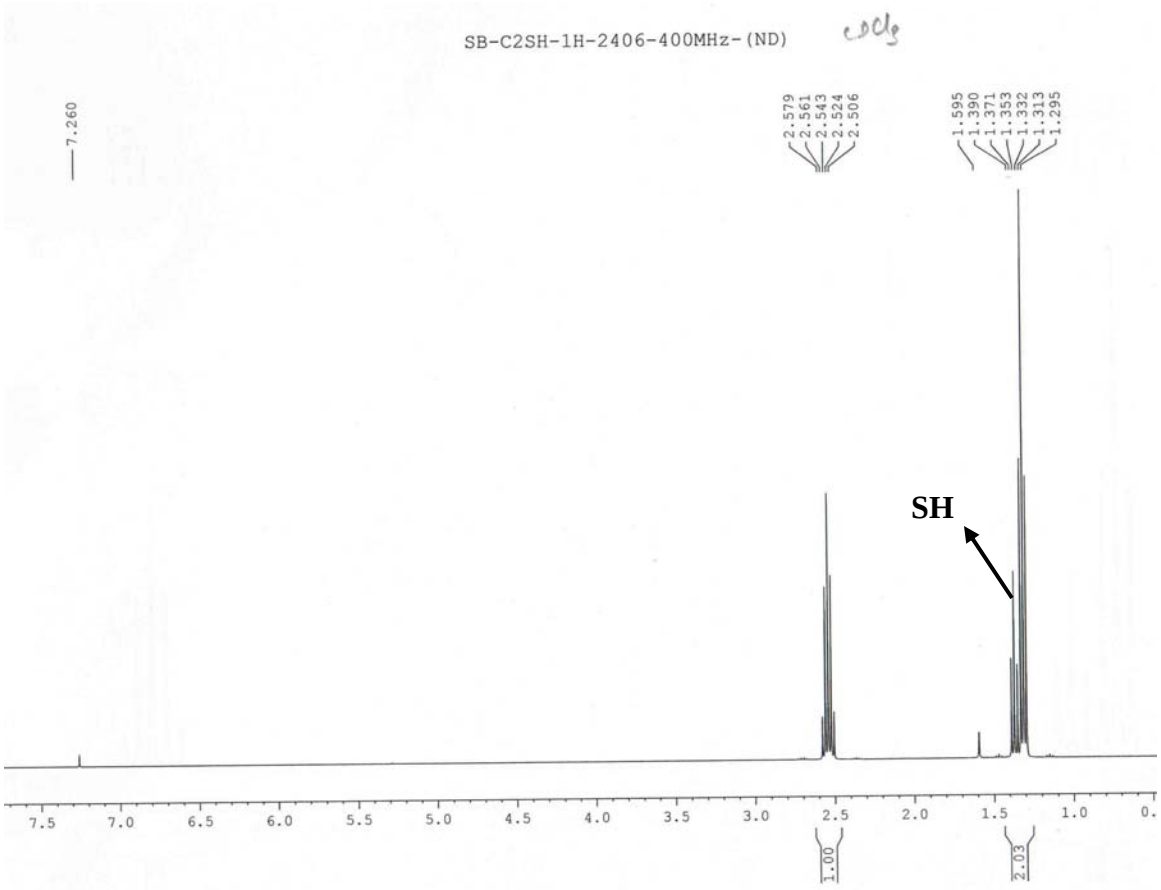

Fig. S4. C_2H_5SH in CD_3OD

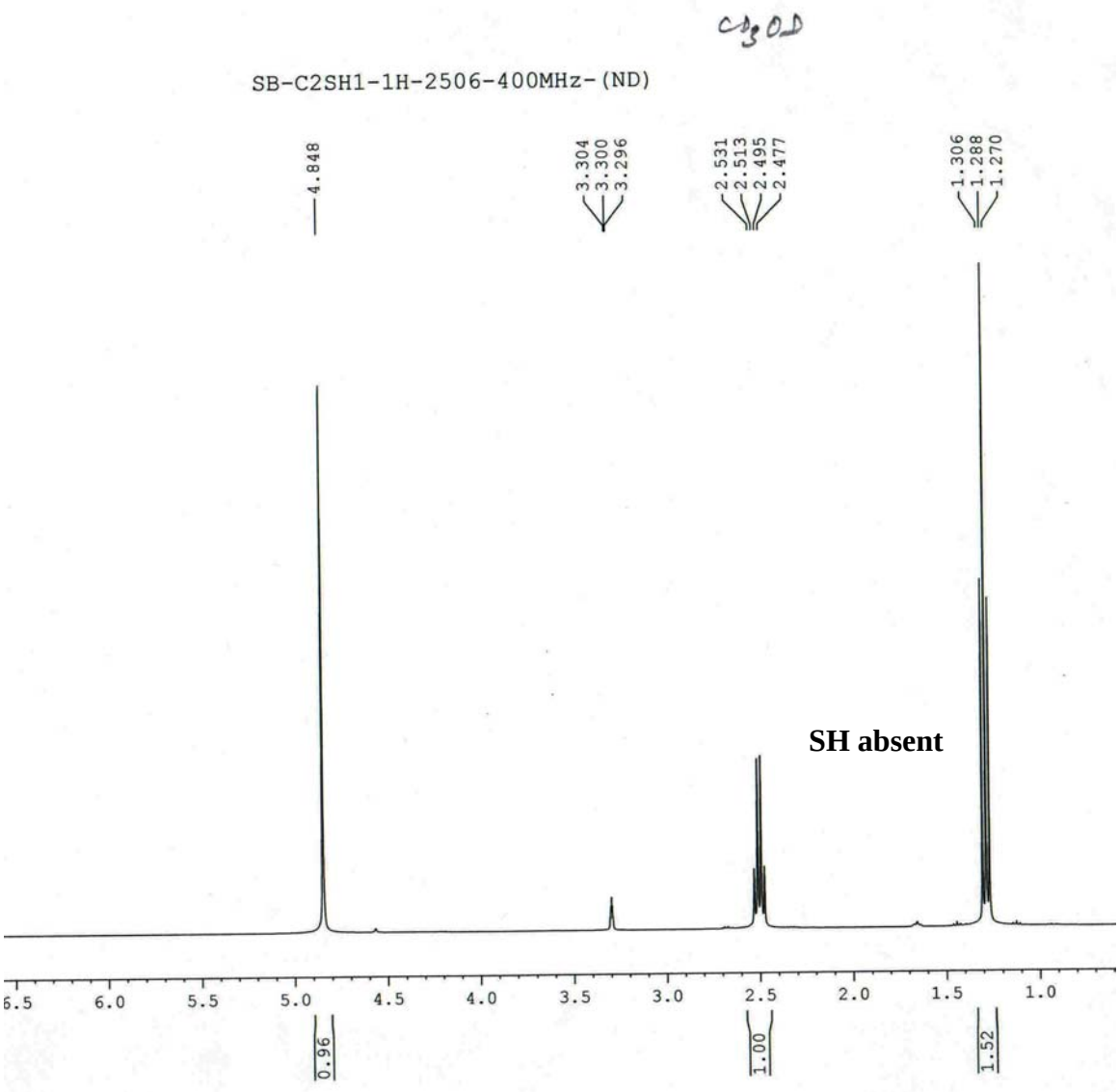

Fig. S5. $C_8H_{17}SH$ in $CDCl_3$

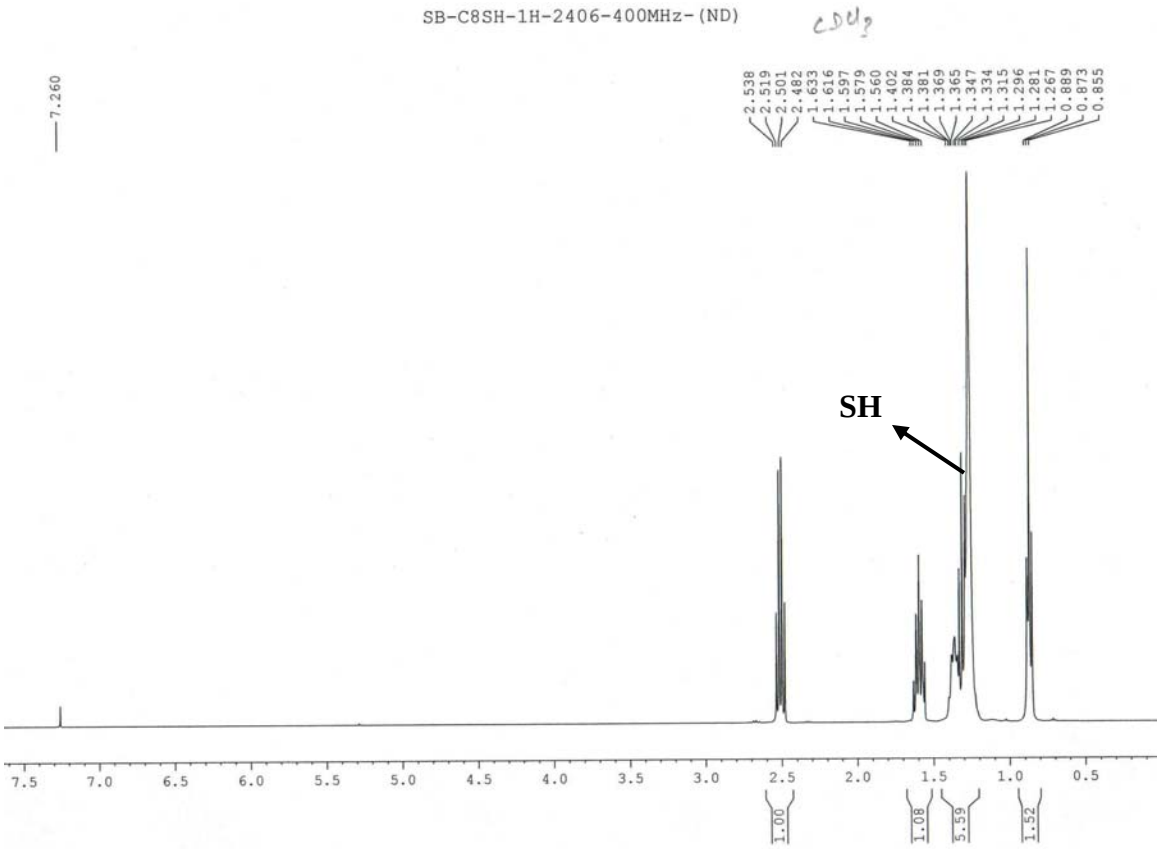

Fig. S6. $C_8H_{17}SH$ in CD_3OD

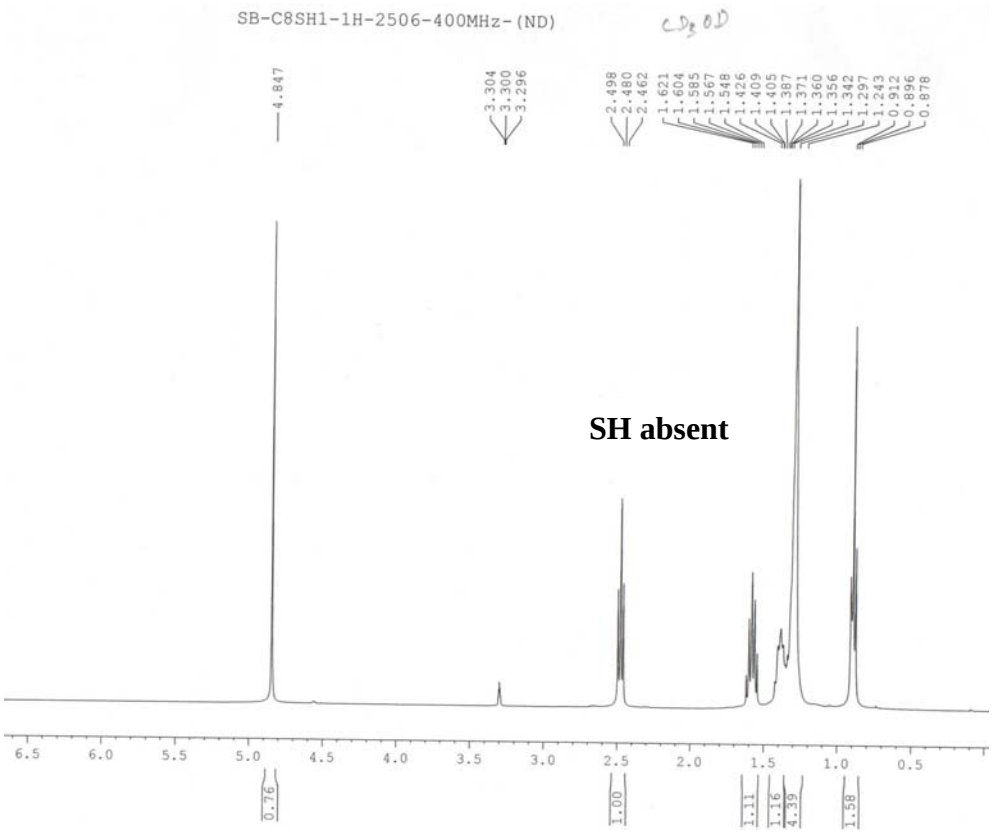

Fig. S7. Glutathione in D_2O

S8-S12: Difference Spectra (Raman) of the thiols

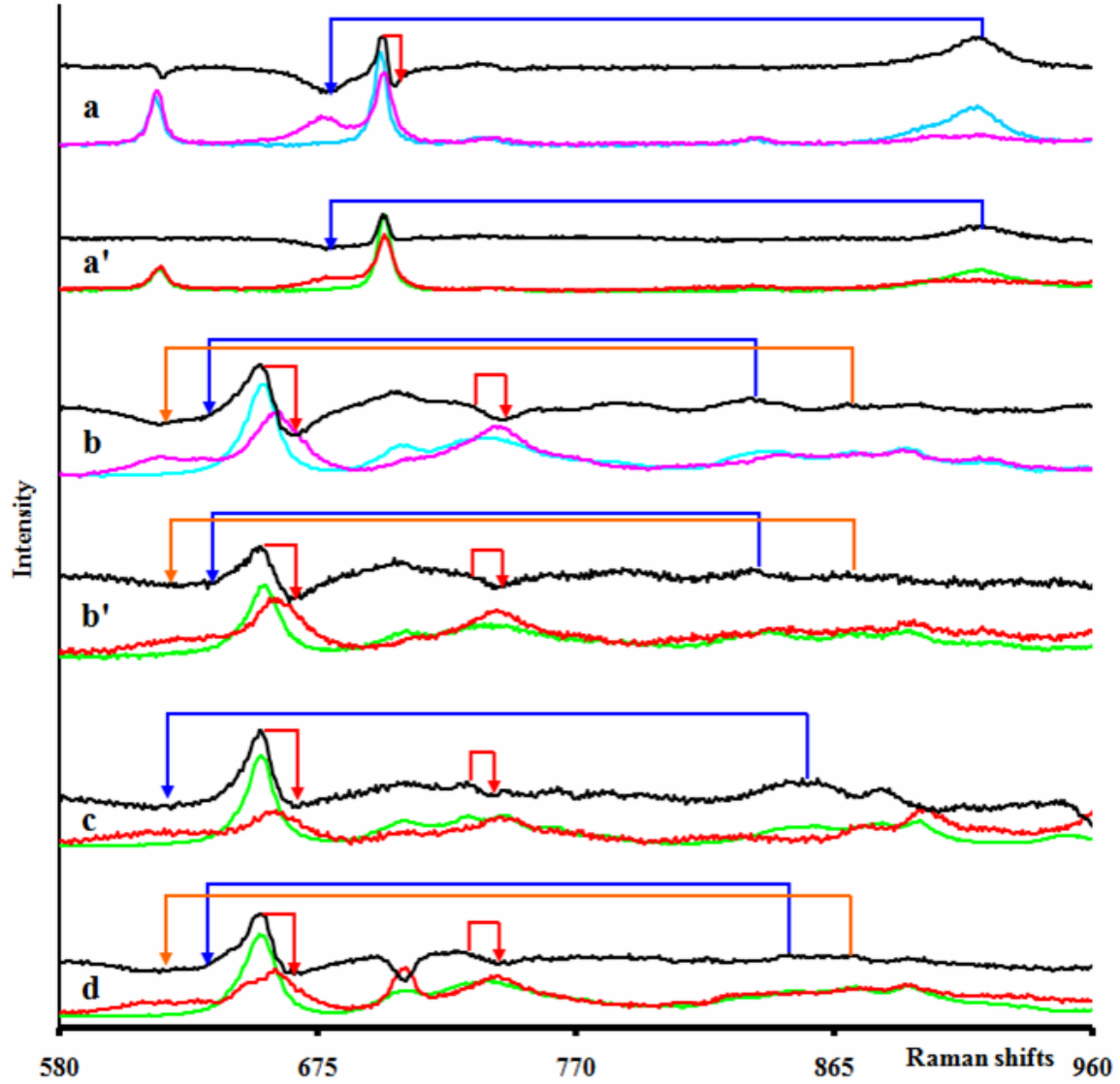

Fig. S8. The Raman spectra of (a) neat PhSH and PhSD; (a') PhSH in CH_3OH and CD_3OD; (b) neat $C_8H_{17}SH$ and $C_8H_{17}SD$ (b') $C_8H_{17}SH$ in CH_3OH and CD_3OD; (c) C_4H_9SH in CH_3OH and CD_3OD and (d) $C_{12}H_{25}SH$ in CH_3OH and CD_3OD. Sky blue and green spectra are for protonated species or solutions and pink and red spectra are for deuterated species or solutions. Black spectra represent the difference spectra of corresponding species. The blue and orange arrows represent the shift of β_{CSH} after deuteration and the red arrow represents the shift of ν_{CS} for the same. Raman shifts are in cm^{-1}.

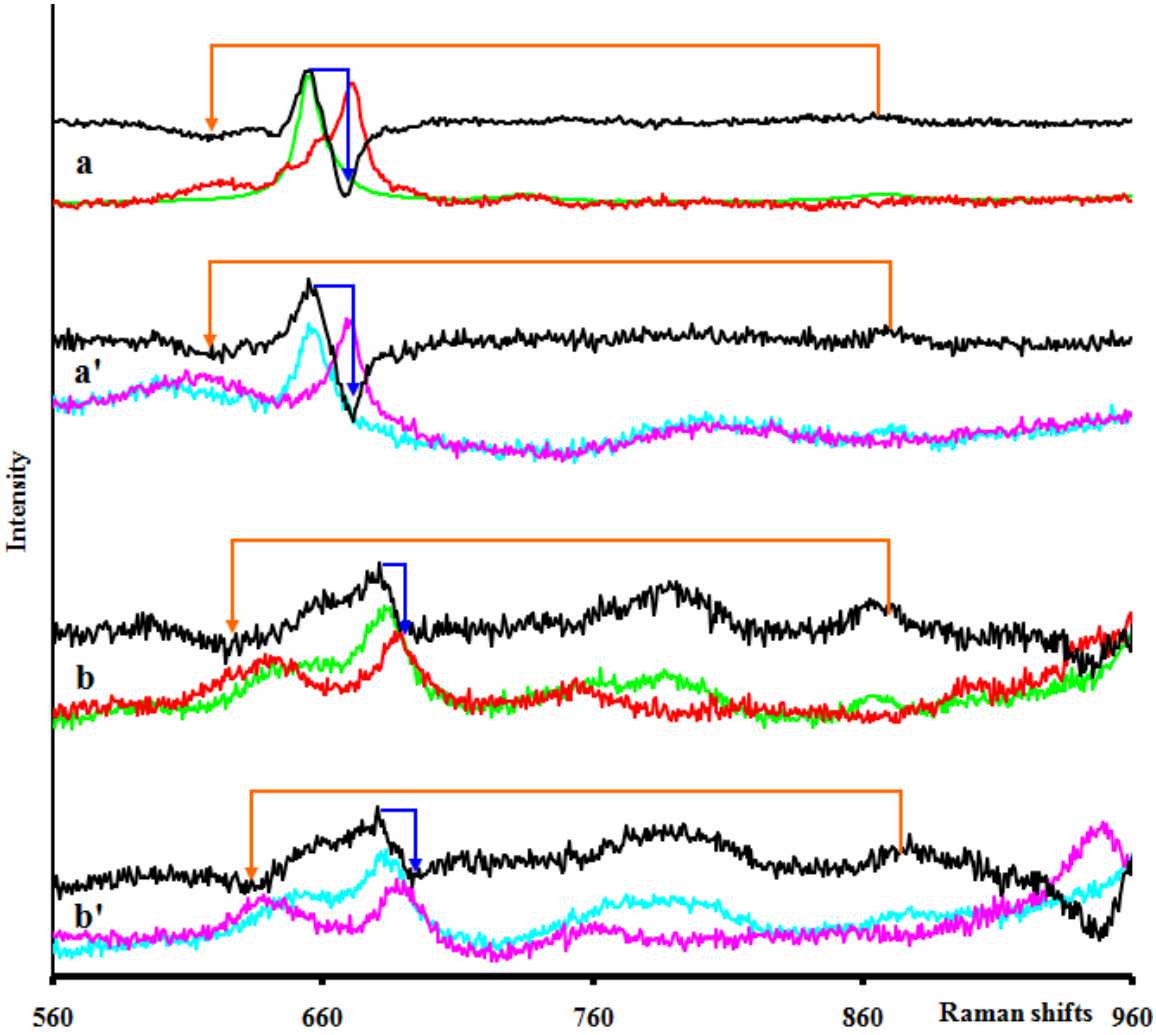

Fig. S9. The Raman spectra of C_2H_5SH in (a) CH_3OH (green) and CD_3OD (red) and in (a') H_2O (sky blue) and D_2O (pink), and of N-acetyl cysteine in (b) CH_3OH and CD_3OD and in (b') H_2O and D_2O (same colour code). Black spectra represent the difference spectra of corresponding species. The orange arrow represents the shift of β_{CSH} after deuteration and the blue arrow represents the shift of ν_{CS} for the same. Raman shifts are in cm^{-1}.

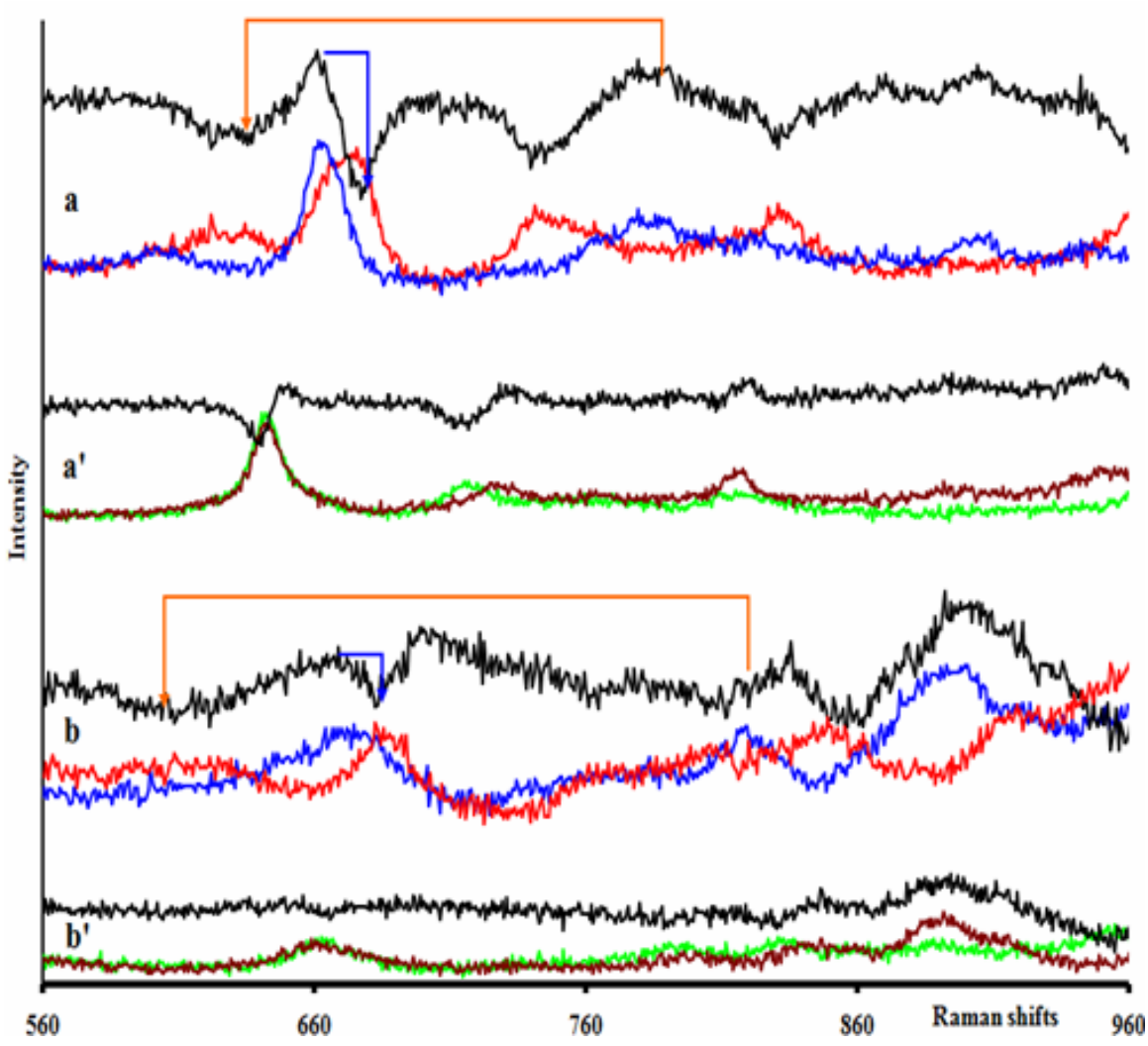

Fig. S10. The Raman spectra of (a) cysteamine, (a') cystamine and of (b) glutathione, (b') glutathione (S-S) solubilized in H_2O and D_2O. Blue and red spectra corresponds to free thiols in H_2O and D_2O, respectively and brown and green spectra corresponds to disulphide in H_2O and D_2O, respectively. Black spectra represent the difference spectra of corresponding species. The orange arrow represents the shift of β_{CSH} after deuteration and the blue arrow represents the shift of ν_{CS} for the same. Raman shifts are in cm^{-1}.

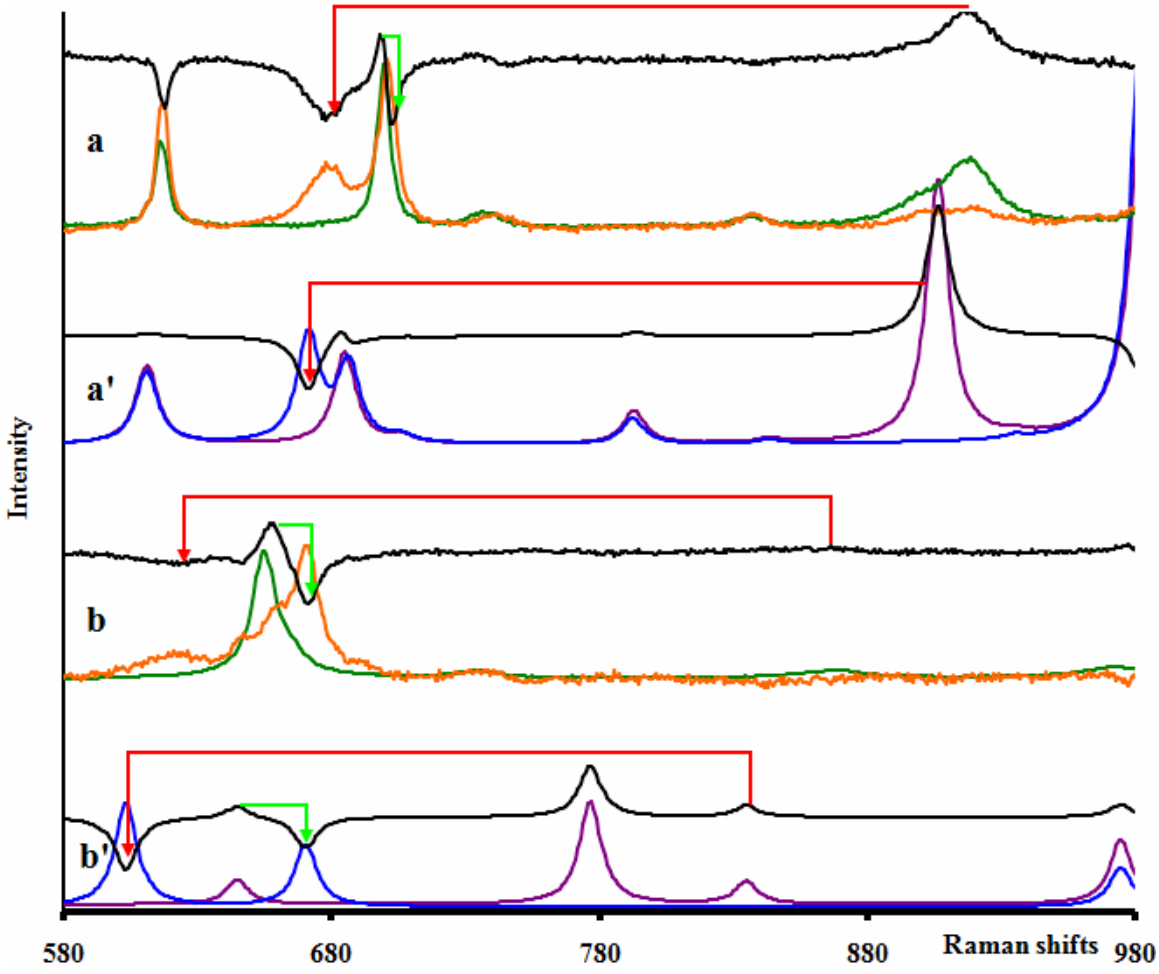

Fig. S11. The Raman spectra of (a) PhSH in CH_3OH (green) and CD_3OD (orange), of (a') PhSH (violet) and PhSD (blue) by theoretical predictions, of (b) C_2H_5SH in CH_3OH (green) and CD_3OD (orange) and of (b') C_2H_5SH (violet) and C_2H_5SD (blue) by theoretical predictions. Black spectra represent the difference spectra of corresponding species. The red arrow represents the shift of β_{CSH} after deuteration and the green arrow represents the shift of ν_{CS} for the same. Raman shifts are in cm^{-1}.

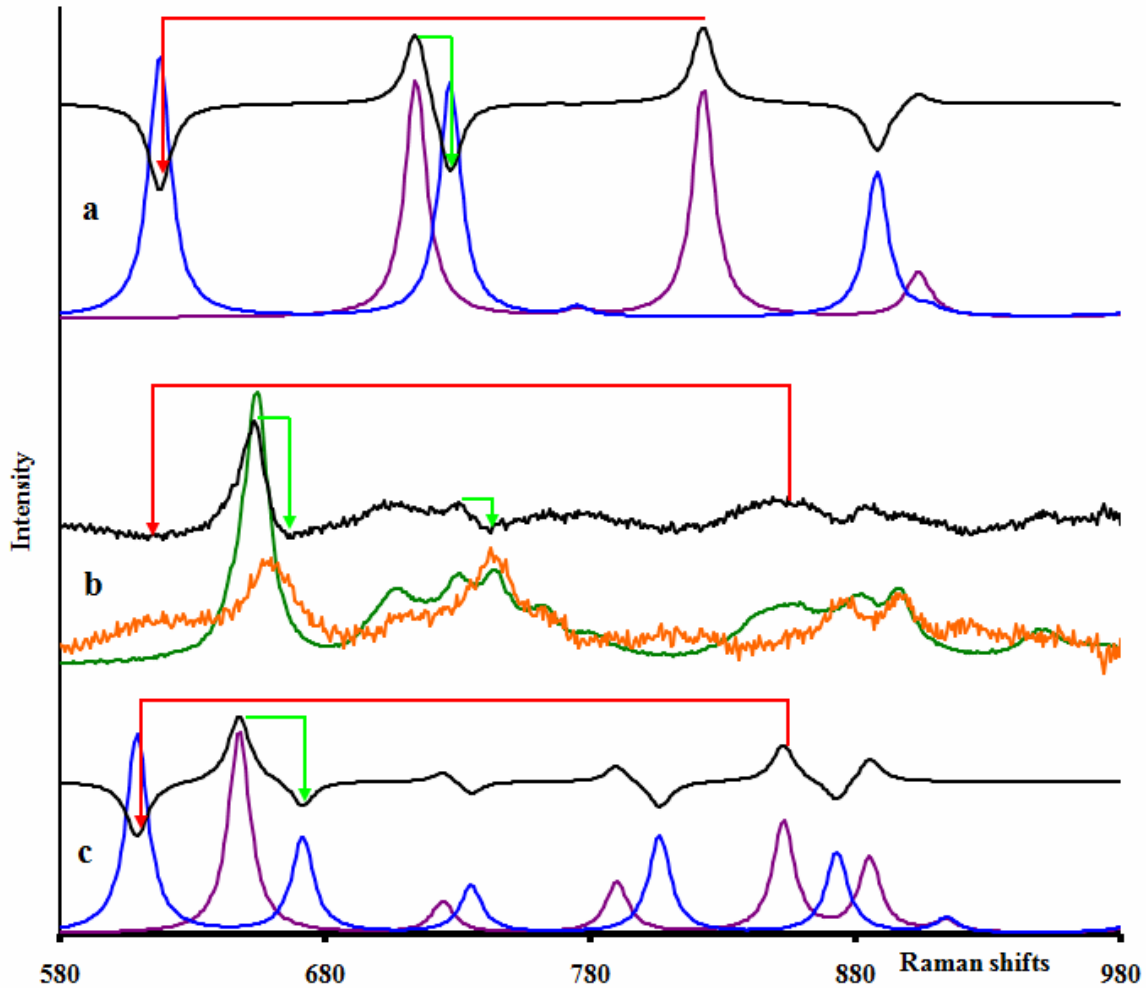

Fig. S12. The Raman spectra of C_4H_9SH in CH_3OH (green) and CD_3OD (orange). Theoretically predicted results in (a) trans conformation and in (c) gauche conformation. In theoretical spectra violet and blue represents the protonated and deuterated results, respectively. Black spectra represent the difference spectra of corresponding species. The red arrow represents the shift of β_{CSH} after deuteration and the green arrow represents the shift of ν_{CS} for the same. Raman shifts are in cm^{-1}.

Table S1. Raman vibrational assignments ofd peak frequencies (cm^{-1}) for neat thiols and for the solutions of thiols in CH_3OH, CD_3OD, H_2O and D_2O.

	Wave number (cm^{-1})				
	Neat		**Solution**		**Assignments**
	SH	**SD**	**CH_3OH**	**CD_3OD**	
	617	617	617	617	ν_{CS}
PhSH	698	698	698	698	ν_{CS}
	918	680	918	680	$\beta_{CSH/CSD}$
			654	661	$\nu_{CS\text{-}G}$
C_4H_9SH			732	745	$\nu_{CS\text{-}T}$
			846,859	614	$\beta_{CSH/CSD}$
	655	661	655	661	$\nu_{CS\text{-}G}$
$C_8H_{17}SH$	739	743	739	743	$\nu_{CS\text{-}T}$
	840,873,893	618,634	840,873,893	630	$\beta_{CSH/CSD}$
			655	660	$\nu_{CS\text{-}G}$
$C_{12}H_{25}SH$			739	742	$\nu_{CS\text{-}T}$
			850,872	612, 632	$\beta_{CSH/CSD}$
	Alcoholic solution		**Aqueous solution**		
	CH_3OH	**CD_3OD**	**H_2O**	**D_2O**	
	654	671	656	671	ν_{CS}
C_2H_5SH	870	622	872	620	$\beta_{CSH/CSD}$
	685	688	682	692	ν_{CS}
N-ac-cysteine	865	638	876	640	$\beta_{CSH/CSD}$
	654	-	650	-	C=O def.

ν indicates the stretching mode, β indicates the in-plane bending mode and def. = deformation.

Table S2. Raman vibrational assignments and peak frequencies (cm^{-1}) of thiols and their corresponding disulfides in H_2O and D_2O.

	Free thiol		**Disulphide**		
	H_2O	**D_2O**	**H_2O**	**D_2O**	
Cysteamine / Cystamine	664	676	642	642	ν_{CS}
	785	632	-		$\beta_{CSH/CSD}$
	785	742	-		sym-NH_2
	900	834	-		asym-NH_2
Glutathione / Glutathione (S-S)			730	719	
	673	689	662	662	ν_{CS}
	820	620			$\beta_{CSH/CSD}$
	820	808	845	835	$\nu_{C\text{-}CN}$
	895	850	895	840	ν_{CC}, $\nu_{C\text{-}COO}^{-}$

ν indicates the stretching mode, β indicates the in-plane bending mode.

Table S3. Theoretically calculated Raman vibrational assignments and peak frequencies (cm^{-1}) of an aromatic thiol and two aliphatic thios in their protonated and deuterated forms.

Wave number (cm^{-1})								
PhSH		**C_2H_5SH**		**C_4H_9SH(T)**		**C_4H_9SH(G)**		
SH	**SD**	**SH**	**SD**	**SH**	**SD**	**SH**	**SD**	**Assignments**
610	610	645	671	715	728	649	673	ν_{CS}
688	688	-		-		-		ν_{CS}
908	672	835	604	823	618	852, 886	611	$\beta_{CSH/CSD}$
-		-		-		725, 790	735, 805	$\beta_{CSH/CSD}$, $\gamma_{CH2/CH3}$
793	793	780	780	-		-		$\gamma_{CH/CH2}$
-		-		904	888	-		$\beta_{CSH/CSD}$ and ν_{CC}
						886	872	$\gamma_{CH2/CH3}$

T and G represent the trans and gauche conformations, respectively, ν indicates the stretching mode, β indicates the in-plane bending mode and γ indicates the rocking mode.

Full Referene for Gaussian 03:

(1) Frisch, M. J. T., G. W.; Schlegel, H. B.; Scuseria, G. E.;; Robb, M. A. C., J. R.; Scalmani, G.; Barone, V.; Mennucci,; B.; Petersson, G. A. N., H.; Caricato, M.; Li, X.; Hratchian, H.; P.; Izmaylov, A. F. B., J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.;; Ehara, M. T., K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima,; T.; Honda, Y. K., O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; ,; Jr., P., J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.;; Kudin, K. N. S., V. N.; Kobayashi, R.; Normand, J.;; Raghavachari, K. R., A.; Burant, J. C.; Iyengar, S. S.; Tomasi,; J.; Cossi, M. R., N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J.; B.; Bakken, V. A., C.; Jaramillo, J.; Gomperts, R.; Stratmann, R.; E.; Yazyev, O. A., A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.;; Martin, R. L. M., K.; Zakrzewski, V. G.; Voth, G. A.; Salvador,; P.; Dannenberg, J. J. D., S.; Daniels, A. D.; O; . Farkas; Foresman, J. B. O., J. V.; Cioslowski, J.; Fox, D. J.**,** *Gaussian 03, C.02* **2004,** Gaussian, Inc.: Wallingford, CT,.