Interesting Nuclear Magnetic Resonance studies of some N, N-bis(2-methoxyethyl) substituted Benzamides

Mamta Sharma[1], Sujeet Kumar Mewar[2]

Abstract

DEET (*N,N* Diethyl m-toluamide) and DEPA (Diethyl phenyl acetamide) are synthetic compounds and proven potent insecticide and repellent respectively. In the search of effective mosquito repellent, different derivatives of DEPA or substituted benzamides were synthesized and their NMR analysis was carried out at low temperature. Sterically Crowded Bis (2-methoxyethyl) substituted Benzamides possess low rotational barrier and are floppy at room temperature. Alkyl arms attached to nitrogen become magnetically nonequivalent even at low temperature. Di ortho Substitution in benzamides enhanced hindered internal rotation and resulted splitting in methylene proton signals. The effect of substitution in benzene ring, on the splitting of methylene proton NMR is very well explained and the complete NMR data of substituted benzamides is described in this study for reference purpose.

Keywords: Proton NMR; Carbon NMR; Heteronuclear Single Quantum Coherence Spectroscopy (HSQC); Rotamers.

Synopsis

Herein, Synthesis, NMR characterization of nineteen substituted benzamides at low temperature and study of formation of rotamers are reported. The results suggest that molecules are floppy in nature and form number of rotamers at room tem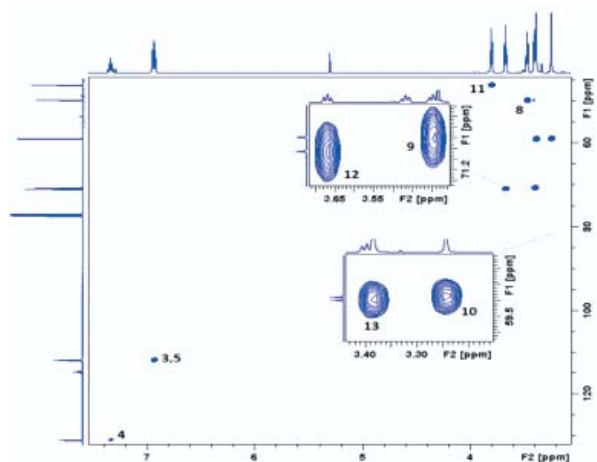perature. The ortho substitution on benzene ring possess restriction in rotation and reduce the formation of rotamers and causes clear splitting of methylene signals on NMR Chemical shift scale.

Author Affiliation: [1]Directorate of International Co-operation, Defence Research and Development Organisation, Raja ji Marg, New Delhi 110011, India, [2]Scientist, Department of NMR, All India Institute of Medical Sciences, New Delhi 110029, India.

Corresponding Author: Mamta Sharma, Directorate of International Cooperation, Defence Research and Development Organisation, Raja ji Marg, New Delhi-110011, India.

E-mail: reekana@rediffmail.com

78

Mamta Sharma, Sujeet Kumar Mewar/Interesting Nuclear Magnetic Resonance studies of some N,N-bis(2-methoxyethyl) substituted Benzamides

INTRODUCTION

Mosquitoes are the major source of vector borne diseases like Malaria, Dengue and Chikungunya etc. Globalization, frequent movement of travelers are one of the causes of these fast spreading diseases. In-spite of lot of development in the field of insect or mosquito repellent, a mosquito free environment still a challenge for developing countries. Its complete eradication seems merely impossible and it has become a horrifying dream for scientific community.[1] Therefore continuous research towards the effective control of population of mosquitoes is a prime requirement of present world. Currently much emphasis is being given on the insect repellent research and taken it as an alternative of the insecticides. This is all because of the toxic effects of the latter on the human being and environment, and being a less toxic, former has potential to apply on skin and to prevent the stored products by repelling insects. In this way insect repellents effectively help to prevent and control the insect borne diseases. In recent past out breaks of chikv fever[2], yellow fever[3] and dengue[4] once again strongly fixed the requirement of mosquito repellents. So far, this field has not been explored fully, hence there is a need to develop new effective insects repellent which should be nontoxic and more environmental friendly. Various insect repellents have been discovered so far like Dimethyl phthalate (1929), Indalone (1937), military formulation 6-2-2, DEET (1953), DEPA (1990), n-methylneo decanamide (1998), 1-piperidine carboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester (Yap *et al.*, 2000). Among these DEET (N, N Diethyl m-toluamide) is a well known effective and versatile mosquito repellent. In this connection, DEPA (Diethyl phenyl acetamide)[5, 6] was reported as cockroach/multi insect repellent long back. Some naturally occurring sources are also reported as repellent to certain insects while they may act as insecticide for others. The natural compound 'Repel Lemon Eucalyptus' has been reported as more active in comparison to synthetic DEET, when the activity of different synthetic, chemical and herbal repellents were compared.[7-9] Most of the reported insect repellents are having benzamide linkages, so keeping this as target, number of benzamide derivatives were synthesized with different substitution in the benzene ring and their efficacy as mosquito repellent is under evaluation.

This paper describes the complete NMR structure elucidation of substituted benzamides, synthesized by simple and easy method. The ^{1}H and ^{13}C NMR assignment of 19 benzamides, having single and double substitution in their aromatic ring presented very interesting changes in splitting pattern of methylene proton signals with substitution in benzene ring. To the best of our knowledge and literature surveyed, no unambiguous NMR assignment for both the nuclei ^{1}H and ^{13}C is available in the literature. These data obtained by a combination of 1D ^{1}H, ^{13}C and 2D ^{1}H-^{1}H Correlation Spectroscopy (COSY), Heteronuclear single quantum coherence spectroscopy (HSQC), Heteronuclear Multiple Bond Correlation Spectroscopy (HMBC), Two Dimensional Nuclear Overhauser Effect Spectroscopy (2D-NOESY) NMR experiments.

EXPERIMENTAL METHODS

2.1 Synthesis

All the substituted benzamides (1-19) were synthesized as shown in scheme 1. Substituted Benzoic acid was dissolved in THF solvent. After this 1, 1' -Carbonyldiimidazole reagent[10] was added to make the inter mediate and kept for 10 to 15 minutes at RT. The evolved gas carbon dioxide during the reaction was removed by applying vacuum using rotavac. Bis(2-methoxyethyl) amine was added to the reaction mixture and kept for stirring for 10 to 15 minutes. Reaction was monitored by TLC and GC up to the completion. Reaction mixture was neutralized with 10% Sodium bicarbonate solution. The organic layer was separated out and THF was
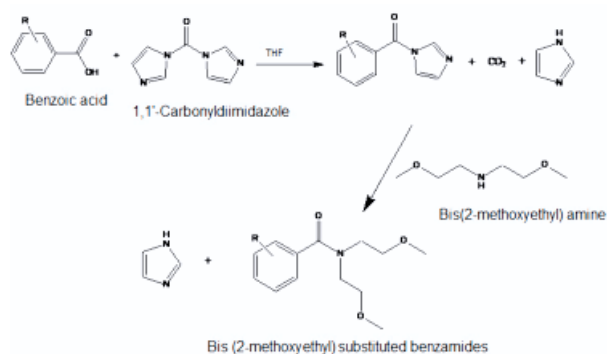
Scheme 1: Synthesis of *N,N*-bis(2-methoxyethyl) substituted benzamides entries (1-19)

removed by vacuum to obtain the desired amide.

2.2 Materials and Physical measurements

The chemical shifts of ^{1}H and ^{13}C nuclei were referenced keeping spectrometer reference frequency at 0 ppm (SR=0). The synthetic Bis(2-methoxyethyl) substituted benzamide derivatives

Mamta Sharma, Sujeet Kumar Mewar/Interesting Nuclear Magnetic Resonance studies of some N,N-bis(2-methoxyethyl) substituted Benzamides

79

1–19 were dissolved in CDCl3 for NMR analysis. Spectra were scanned by taking 80 µl concentration of compounds in the 500 µl of solvent in 5 mm wilmad quartz NMR tube. All the NMR data were recorded on a Bruker Avance III 600 MHz spectrometer system (14 T; Ultra shield plus, Bruker Germany) at topspin 3.1 version and temperature range 288 to 293K using QNP cryoprobe. The ^{1}H NMR experiments were carried out by keeping relaxation delay 1 sec., spectral width 20ppm, and 90° pulse 5.25 µsec., pulse programme zg30, and fid resolution 0.366 Hz. For the ^{13}C NMR experiments the relaxation delay, spectral width and 90° pulse were kept 2s, 239 ppm, and 9.47 µsec. respectively. 2-dimensional experiments including COSY, HMBC were performed by acquiring 2048 data points for t2 and 128 data points for t_1 while in HSQC t_2 and t_1 were adjusted at 1024 and 256 respectively. The long range coupling time for HMBC was 70 ms prior to Fourier transformation, zero filling of 2k and a sine squared bell window function were applied using topspin model 3.1. *Noesy* experiments were performed with 16 acquisitions for 256 increments in F1 and 1024 data points in F2. The spectral width in both dimensions was 10 ppm. Gradient based Phase sensitive noesys pulse program was used with mixing time 80 msec to 300 ms, 2s relaxation delay at temp 290° K.

RESULTS AND DISCUSSION

Amides have been studied considerably, more effectively, by the use of Nuclear Magnetic Resonance Spectroscopy. It was realized that unambiguous ^{1}H and ^{13}C NMR assignment should be available in the literature for substituted benzamides, synthesized in laboratory. The structure of all the compounds and numbering used in proton and carbon nuclei assignment are shown in scheme 2. Resonance assignment of ^{1}H and ^{13}C is done based on the multiplicity pattern, δ and J, which is further confirmed by 2D NMR experiments like COSY, HSQC, HMBC and NOESY.
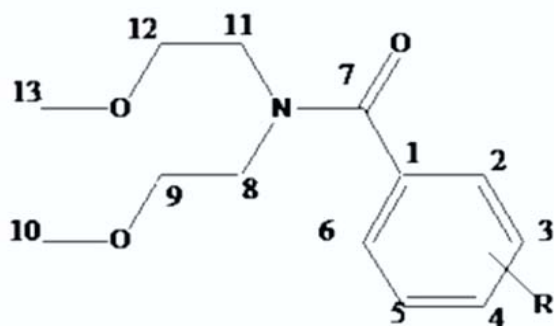
Entry	R*	Entry	R*
1	0	11	2,6-OMe
2	4-Cl	12	3,4-OMe
3	2-OMe	13	2,6-F
4	3-OMe	14	2,3-F
5	4-OMe	15	2,4-F
6	2-F	16	2,5-F
7	4-F	17	3,5-F
8	2,6-Cl	18	2-NO2
9	3,5-Cl	19	4-NO2
10	3,4-Cl		

R* = Substitution on Benzene Ring

Scheme 2. Structure of *N,N*-bis(2-methoxyethyl) Substituted Benzamides (1-19).

Proton NMR

The full characterization of benzamides containing chemical shift (δ) of ^{1}H nucleus is shown in the Table 1 and coupling constant (J) of ^{1}H nucleus is given in Supplementary information. The ^{1}H NMR spectra of entries 1-19 showed clearly resolved two distinct regions, temperature independent aromatic region and temperature dependent aliphatic region with $A_2B_2C_2D_2$ spin system for methylene protons. Entries 3/18 presented $A_2BB'CC'D_2$ /A$_2$B$_2$CC'DD' systems for alkyl protons respectively. As far as the proton NMR spectrum of precursor Bis 2-methoxyethyl amine is concerned, this exhibited single peak for two methoxy groups at δ 3.36 ppm, while this signal further segregated into two for protons of both the methoxy groups after the formation of amide, resulting shifting of one signal towards the high field and other shifts at low field. In the similar manner, both the methylene protons attached to nitrogen and oxygen exhibited same δ value at 2.80 ppm and 3.50 ppm for protons of N-$(CH_2)_2$ and $(O-CH_2)_2$ groups respectively in amine, but these appeared as four well resolved signals, representing four separate methylene groups of aliphatic chain, in benzamides (Table 1, Fig. 2). This confers that protons of all four methylene groups of both the aliphatic chains attached to nitrogen and oxygen become magnetically different after formation of benzamides. In some cases this separation was observed at low temperature. This is well documented that internal rotation around single bond of amide (CO-N) is some what restricted due to partial double bond character of bond and this rotation is affected by various substitutions of amide nitrogen and carbon. The internal rotation around the bond normally occur via two transition states; anti TS_1 or syn TS_2 and causes rotamers formation in amides.[11-23]

Mamta Sharma, Sujeet Kumar Mewar/Interesting Nuclear Magnetic Resonance studies of some N,N-bis(2-methoxyethyl) substituted Benzamides

80

Table 1A: ¹H NMR Chemical shifts of *N,N*-bis (2-methoxyethyl) Substituted Benzamides for entries 1 to 19 in ppm. (for 2H to 6H Protons)

Entry	R	2H	3H	4H	5H	6H
1	-	7.41 m	7.38 m	7.39 m	7.38 m	7.41 m
2	-	7.40 d	7.39 d	-	7.39	7.40
3	3.83 s	-	6.91 dd	7.33dt	6.98 dt	7.22 dd
4	3.84 s	6.98 t	-	6.93 ddd	7.31 t	6.99 td
5	3.85	7.42 d	6.92 d	-	6.92 d	7.42 d
6	-		7.06 ddd	7.32 dt	7.15 dt	7.34
7	-	7.42 dd	7.04 t	-	7.04 t	7.42 dd
8	-		7.33 d	7.26 dd	7.33 d	-
9	-	7.34 d	-	7.37 t	-	7.34 d
10	-	7.58 d	-	-	7.48 d	7.31 dd
11	3.82	-	6.58 d	7.28 t	6.58 d	-
12	3.87, 3.88	7.06 d	-	-	6.84 d	7.03 dd
13	-	-	6.93 dd	7.34 tt	6.93 dd	-
14	-	-	-	7.18 dtd	7.12 dt	7.11m
15	-	-	6.83 dt	-	6.90 dt	7.33 dt
16	-	-	7.05 m	7.06 m	-	7.07 m
17	-	7.00 m	-	6.85 tt	-	7.01 m
18	-	-	8.22 dd	7.57 dt	7.72 dt	7.47 dd
19	-	7.58 d	8.21 d	-	8.21 d	7.58 d

S: singlet, m: multiplet, t: triplet, td: triplet of doublet, d: doublet, dd: doublet of doublet, ddd: doublet of doublet of doublet, tt: triplet of triplet, dt: doublet of triplet, dtd: doublet of triplet of doublet.

Table 1B: ¹H NMR Chemical shifts of *N,N*-bis(2-methoxyethyl) Substituted Benzamides for entries 1 to 19 in ppm. (for 8H to 13H Protons)

Entry	8H	9H	10H	11H	12H	13H
1	3.54,bs	3.44,bs	3.28,s	3.78,bs	3.67,bs	3.41,s
2	3.53,bs	3.45,bs	3.31,s	3.76,bs	3.68,bs	3.40,s
3	3.40,bs	3.39,3.35,bs	3.23,s	3.74,3.82,bs	3.68,t	3.41,s
4	3.55,bt	3.46,bt	3.30,s	3.77,bt	3.69,bt	3.41,s
5^	3.59,bs	3.47,bs	3.31,bs	3.76,bs	3.68,bs	3.40,bs
6	3.44,t	3.36,t	3.20,s	3.78,bs	3.64,t	3.35,s
7	3.49,bs	3.41,bs	3.24,s	3.71,bs	3.63,bs	3.34,s
8	3.43,t	3.47,t	3.29,s	3.85,t	3.73,t	3.40,s
9	3.51,bt	3.45,bt	3.30,s	3.74,bt	3.66,bt	3.39,s
10	3.52,s	3.45,s	3.30,s	3.74,s	3.66,s	3.39,s
11	3.39,t	3.38,t	3.25,s	3.80,t	3.70,t	3.42,s
12^	3.58,bs	3.47,bs	3.29,bs	3.71,bs	3.65,bs	3.36,bs
13	3.47,t	3.40,t	3.24,s	3.78,t	3.67,t	3.38,s
14	3.46,t	3.40,t	3.23,s	3.77,bs	3.66,t	3.38,s
15	3.44,t	3.37,t	3.22,s	3.75,bs	3.62,t	3.36,s
16	3.49,t	3.42,t	3.27,s	3.78,bs	3.67,t	3.39,s
17	3.52bs	3.46,bs	3.31,s	3.75,bs	3.67,bs	3.39,s
18	3.41,bs	3.42,bs	3.26,s	3.96,3.63,bs	3.74,3.82,bs	3.44,s
19	3.44,bt	3.39,bt	3.24,s	3.73,bt	3.64,bt	3.35,s

bs=broad signal, bt=broad triplet, ^=broad merged six single peaks with joined base at 10ºC t=triplet.

Mamta Sharma, Sujeet Kumar Mewar/Interesting Nuclear Magnetic Resonance studies of some N,N-bis(2-methoxyethyl) substituted Benzamides

81

Plethora of papers witnessed that the barrier of rotation is governed by various factors like solvent polarity[24-30] temp,[20,31-33] molecular phases,[20,26] substitution on carbonyl Carbon,[20,34-39] amide nitrogen and carbine of benzene ring attached to carbonyl.[24,33] Reports deduce that to attain the optimum possible stable ground state geometry, amides possess twist around the C_{ar}-C(O),[40] C(O)-N[41] and N-CR[40,41] bonds. The steric repulsion between alkyl substitutes of amide nitrogen further destabilizes moleculeand lead to frequent changes in alkyl chain geometry with respect to the benzene ring plane. In our case N, N-dialkyl benzamides are sterically crowded, having long alkyl chain and bulky substitutions on benzene ring, hence these molecules always try to adopt the twisted confirmation (Fig. 1a) by twisting C_{ar}-CO and N-CR bond axis.[42] This is clearly visible by changing splitting pattern of proton signals of methylene groups with placement of substitution in benzene ring at low temperature.

In all benzamides, entries 1 to 19, proton signals of two methylene groups attached to nitrogen (N-$(CH_2)_2$) (H-8 and H-11, δ range is 3.38-3.59 ppm and 3.63-3.96 ppm respectively) are more deshielded in comparison to the proton signals of their respective adjacent partners, two methylene groups attached to oxygen (O-$CH_2)_2$ (H-9, and H-12, δ range is 3.35-3.47 ppm and 3.62-3.82 ppm respectively) and appeared at low field always (Table 1). This low field shifting of N-$(CH_2)_2$ group may be attributed to the carbonyl group adjacent to Nitrogen.

Cis-trans

Moreover within the two N-CH_2 groups, H-8 protons are always shielded by 0.13ppm to 0.42 ppm than the H-11 protons in all the entries (Scheme 2, Table 1). The available literature reveals that protons anti to carbonyl oxygen are always shielded in benzamides.[34-38,43,44] The literature values of protons of different substituted group of benzamides are given in supplementary information. Our observation is congruent with the aforementioned findingsof literature. H-8, trans to carbonyl group is shielded than H-11 [Cis to C(O)] for all the entries. The magnitude of shielding of H-8 protons is high for mono and di ortho substituted benzamides than other entries, which is 0.42 ppm (entries 8 & 11), 0.39 ppm (entries 18 & 3), 0.34ppm (entry 6) and 0.30 ppm (entries 13 to 16). (Table 1). The preponderance of the conformer in solution is further suggested by 2D NOESY experiment at 20°C in $CDCl_3$ solvent. Which established that H-8 and H-9 methylene protons remain away from carbonyl group and hence near to protons of benzene ring in space. The 3D spatial hydrogen interaction provided by 2D NOESY is shown in Fig. 1b, exhibiting correlation between H-8/H-9 and H-6/ H-3 protons respectively.
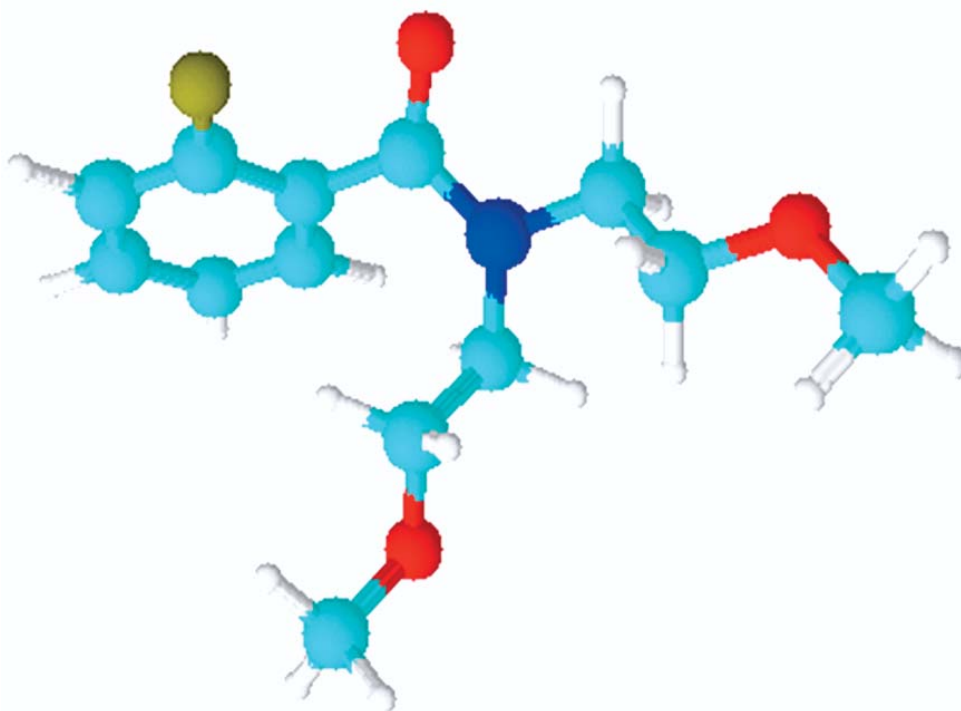

Fig. 1a: 3D Structure of 2-fluoro-*N*,*N*-bis (2-methoxyethyl)benzamide (entry-6) showing connectivity of protons in Space

82

Mamta Sharma, Sujeet Kumar Mewar/Interesting Nuclear Magnetic Resonance studies of some N,N-bis(2-methoxyethyl) substituted Benzamides
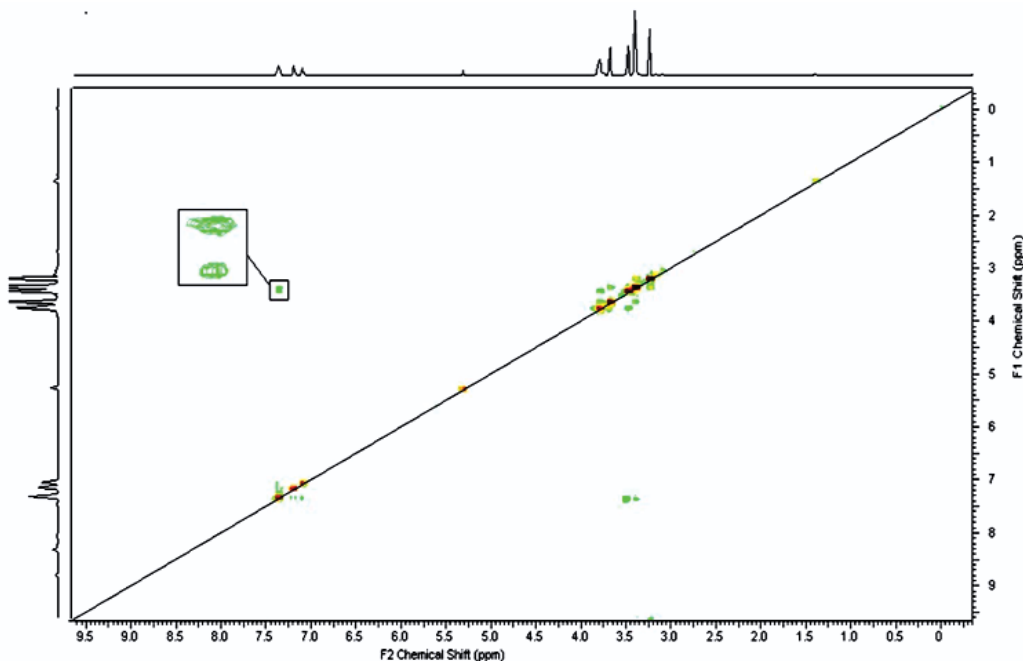
Fig. 1b: H-¹H, 2D-NOESY Spectrum of Benzamide entry 6 Showing Connectivity of H8/H9 with H-6.

Chemical shift sequence of aliphatic chain

Now discussion is made on the δ sequence pattern of methylene and methyl signals on the chemical shift scale, the sequence in which these are appeared at NMR Chemical shift scale. In general proton δ of most of the benzamides (entries 1, 2, 4, 5, 6, 7, 9, 10, 12, 13, 14, 15, 16, 17, and 19) methylene and methyl protons appeared in the order NCH_2 (H-11), OCH_2 (H-12), NCH_2 (H-8), OCH_2 (H-9), OCH_3 (H-13) and OCH_3 (H-10) starting from low field to high field. Under lined fonts are shown to differentiate one aliphatic chain from other in benzamides. The chemical shift sequence of protons is clearly demonstrated in the graph (Fig. 2), where δ values of methylene and methyl protons are plotted against their respective entries. This clearly demonstrated the δ trend, in which H-8, H-9 and H-10 of one alkyl chain are more shielded than H-11, H-12 and H-13 of another alkyl chain, when compared vis a vis with in molecule (Graph, Fig. 2). This distinctly shows that one alkyl arm, cis to the carbonyl oxygen is completely deshielded than the other arm, trans to carbonyl. Di ortho substituted chloro benzamide entry 8, follows slight variation in chemical shift sequence, which is H-11, H-12, H-9, H-8, H-13and H-10 towards high field, where H-8 (δ 3.43 ppm) is slightly shielded than H-9 (δ 3.47 ppm) (Table 1). Entries 3, 11, 18 showed different patterns and exhibited complete true shielding of one arm (H-8, H-9, H-10) than other (H-11, H-12, H-13) irrespective of methyl and methylene groups. H-8 (3.40 ppm), H-9 (3.39 ppm),

H-10 (3.23ppm) protons of benzamide entry 3 are shielded than their respective H-11, (δ 3.77ppm) H-12, (δ 3.68 ppm) and H-13 (δ 3.41 ppm) protons. Similarly H-8 (3.39ppm), H-9 (3.38 ppm), H-10 (3.25ppm) of benzamide entry 11 are more shielded than their respective H-11, (δ 3.80ppm) H-12, (δ 3.70ppm) and H-13 (δ 3.42 ppm) protons. Entry 18 follows δ sequence pattern H-11, H-12, H-13, H-9, H-8, and H-10 towards high field, where H-8 (δ 3.41ppm) protons are slightly shielded than H-9 (δ 3.42 ppm) by 0.01 ppm. These all molecules are having bulky substitution at ortho position that is methoxy (entries 3, 11), chloro (entry 8) and nitro (entry 18) as mono and di substitution. Hence presence of the bulky substitution at ortho position brings variation in chemical shift sequence of methylene and methyl protons of aliphatic chain.

Splitting of Proton Signals

The broadening and splitting of the proton signals of methylene group is dependent on the rotation around the C_{ar}-C(O), C(O)-N, and N-C_{alkyl} bonds and substitutions in amide benzenering. All the benzamides showed broadening/hump for the proton signals at room temperature therefore NMR spectra were recorded at low temperature ranging from 20°C to 10°C for all the compounds and resulted splitting and separation of signals for methylene protons of benzamides. Ortho substituted benzamides exhibited clear splitting/distorted triplet for methylene proton signals which is generated due to hindered rotation, while
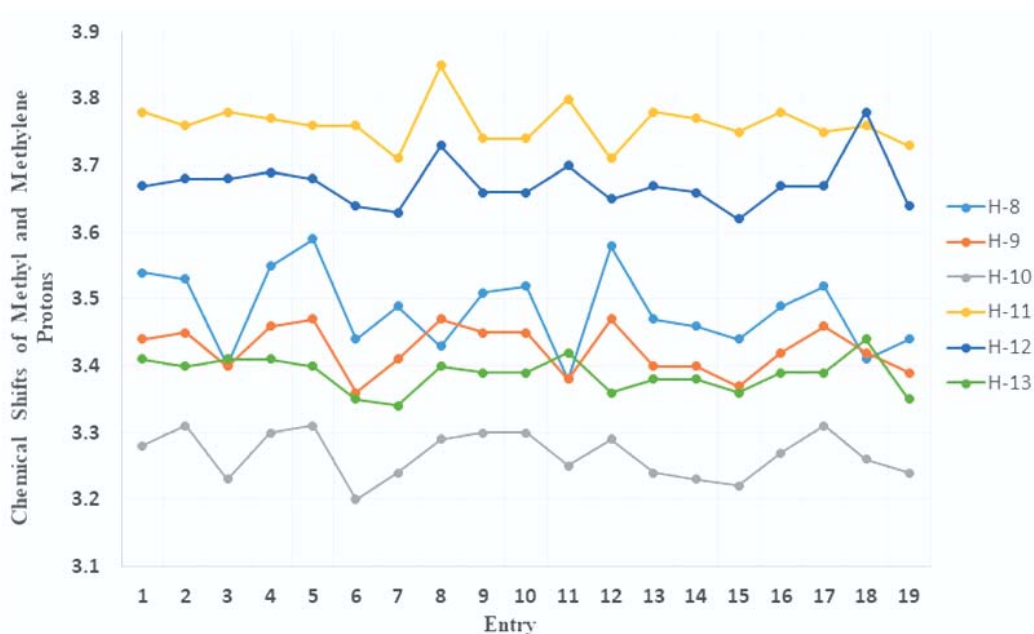
Mamta Sharma, Sujeet Kumar Mewar/Interesting Nuclear Magnetic Resonance studies of some N,N-bis(2-methoxyethyl) substituted Benzamides

83

Fig. 2: Graph depicting the chemical shift trend in methyl and methylene protons in entries 1 to 19

for other pera/meta substituted molecules, unsplit singlets were obtained even at 10°C for methylene protons. This shows that barrier of rotation for ortho substituted benzamides is higher than the pera/ meta substituted benzamides in the temperature range 15°C to 20°C, which leads to the more rotamers formation for pera/meta substituted benzamides and resulted broad humps/singlets for methylene proton signals on NMR Scale. On the basis of available literature data (given as supplementary information) on free energies of activation of the amide bond rotation around (CO)-N bond for the amides/benzamides,[20,34-48] it can be concluded that at RT the barrier of rotation decreases with the increasing size of alkyl groups on nitrogen and carbonyl carbon of amide functionality[20,49] which leads to destabilization at ground state, intern facilitate the rotamers formation fast, and causes broadening of signals also. But on increasing the bulkiness at the ortho position of phenyl ring the barrier of rotation is increased around the amide bond and rotation becomes restricted which leads to the stabilization and facilitate the clear splitting. Therefore benzamides with di ortho, mono ortho, and no ortho substitution showed clear triplets (due to restricted rotation), incomplete splitting pattern (due to intermediate rotation), and unsplit signals (due to fast rotamer formation) for methylene proton signals respectively (Fig. 3). The electron with drawing/donating nature of substituents and their position on benzene ring influence the splitting of methylene proton signals

also. The ^{1}H spectra showing variation in NMR signals with temperature is given in supplemenatry information. After examining the proton spectra of all compounds meticulously it was concluded that Di ortho substituted benzamides entries 8, 11 and 13 presented very clear splitting pattern with little roofing, for protons of all four CH_2 groups. The proton spectra of mono ortho fluorine substituted benzamides entries 6, 14, 15 and 16 appeared as 1+3 pattern (one unsplit signal + 3 split signals) i.e. one CH_2 (H-11) as broad signal while other three CH_2 (H-8, H-9, H-12) appeared as distorted triplet as shown in the fig. 3. Molecule 3 with strong electron donating methoxy group at ortho position exhibited 3+1 (unsplit/ split signals) pattern. Splitting was observed only for H-12 (3.68 ppm), while both the geminal protons of H-9 (3.393, 3.353 ppm) and H-11 (3.74, 3.82 ppm) exhibited two separate δ with broad signals. Mono ortho nitro substituted benzamide 18 presented broad resonances for all the methylene protons and separation in resonances of geminal protons of H-11 and H-12 appeared at δ 3.968, 3.637 ppm and 3.74, 3.82 ppm respectively. Rest of the benzamides 1, 2, 4, 5, 7, 9, 10, 12, 17 and 19 showed unsplit broad singlets for all protons of methylene groups. Hence when rotamers formation fast and slow on NMR chemical shift scale the broadening and splitting of signals occur respectively. The three bond [1]H-^{1}H coupling for methylene protons was found 5.5 Hz in distorted triplets of benzamides. Similar type coupling magnitude in X-CH_2-CH_2-Y type systems is reported.[50]

84

Mamta Sharma, Sujeet Kumar Mewar/Interesting Nuclear Magnetic Resonance studies of some N,N-bis(2-methoxyethyl) substituted Benzamides

After overlapping the aromatic regions of pera substituted benzamides 2 and 5 (given as supplementary information) it was concluded that chlorine exerts more deshielding on ring protons H-3/5, than fluorine. (Table 1).
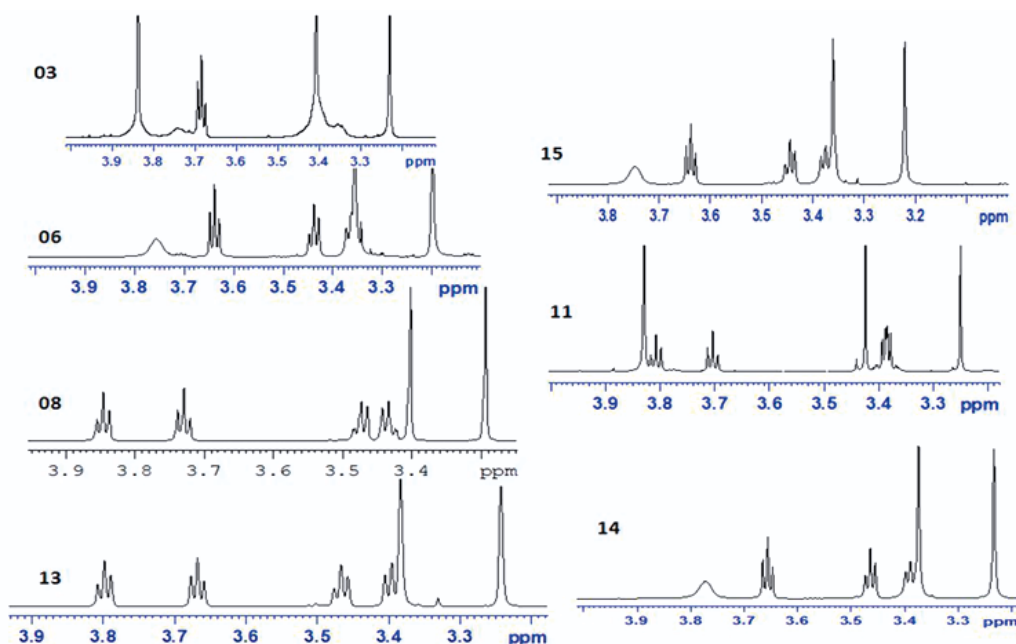

Fig. 3: Expanded ^{1}H NMR Spectra of *N,N*-bis(2-methoxyethyl) substitued benzamides for entries 3,6,8,11,13,14,15, in CDCl$_3$ showing spitting patterns of methylene protons in the chemical shift range 3 to 4 ppm.

42D-COSY

The three bond proton connectivity for protons of N-CH$_2$ and O-CH$_2$ is confirmed by 2D-^{1}H-^{1}H COSY (Homonuclear Correlation Spectroscopy) NMR experiment (Fig. 4), which deduced that adjacent proton signals are of the adjacent methylene groups and coupling with each other. Protons H-11/H-8 are coupling with H-12/H-9 protons respectively and showing correlation contours.
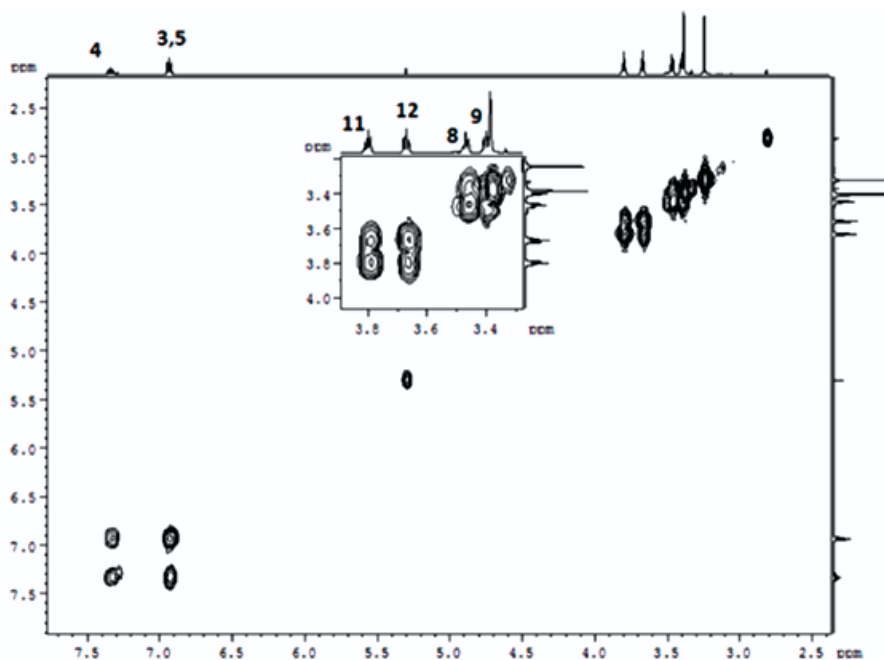

Fig. 4: ^{1}H-^{1}H COSY Spectrum of entry 13 showing three bond Connectivity for adjacent protons of methylene groups in CDCl$_3$

Mamta Sharma, Sujeet Kumar Mewar/Interesting Nuclear Magnetic Resonance studies of some N,N-bis(2-methoxyethyl) substituted Benzamides

85

Carbon NMR

The full characterization of compounds containing chemical shift (δ) of $^{13}C\{^1H\}$ nucleus is shown in the Table 2 and coupling constant (J) is given in 2b table. The interpretation of ^{13}C NMR signals of *N,N*-Bis-(2-methoxyethyl) substituted benzamide entries 1 to 19 was done with the aid of 2D heteronuclear correlation (HSQC) experiment, which allowed the unambiguous assignments of all protonated carbon resonances. For instance the carbon resonances of all the methoxy groups can easily be assigned in the range 55.34–55.90 ppm, as well as the aromatic and aliphatic carbon resonances at 103.95-130.86 ppm and 44.98-71.30 ppm respectively (Table 2). Chemical shift of carbonyl carbon of all the compounds shifted high field in comparison to carbonyl carbon of unsubstituted benzamide entry 1 (Table 2). This chemical shift varies from 0.05 to 10.13 ppm, in all benzamides, while this variation is 3 to 10 ppm in mono and di ortho substituted compounds. Further high range 5 to 10 ppm shift belongs to only halo substitution. The maximum 10 ppm and minimum 0.05 ppm shift was obtained for entries 13 (ortho di fluoro) and 4 (pera-methoxy) respectively (Table 2). Hence it can be concluded that Carbonyl carbon is more shielded in weakly ring deactivating halo ortho substituted benzamides in comparison to the strong electron donating/ with drawing substituents, irrespective

to their position in benzene ring (o/p/m). Proton and carbon single bond correlation is elaborated with the help of HSQC (Heteronuclear single quantum correlation experiment) experiment. Aliphatic carbon resonances C-8 to C-13 (δ range 44.98 ppm–71.30 ppm) presented correlations with H-8 to H-13 Proton resonances (δ range 3.968 ppm–3.20 ppm). This helped in sequencing the resonances of protons and carbons on chemical shift scale. To understand the single bond correlation of proton with carbon, HSQC spectrum of benzamide 13 is shown in Figure 5 as example, which emphasizes the correlation of C-10, δ 58.84 ppm / C-13, δ 58.94 ppm with H-10, δ 3.24 ppm / H-13, δ 3.38 ppm respectively. Simultaneously this established the other correlations also like C-11, δ 45.97 ppm /C-12, δ 70.85 ppm with H-11 δ 3.78ppm/H-12, δ 3.67 ppm and C-8, δ 49.58 ppm /C-9, δ 70.58 ppm with H-8, δ 3.47 ppm /H-9, δ 3.40 ppm. Aromatic protons H-3/5 δ 6.93 ppm, H-4, δ 7.34 ppm correlated with C-3/5, δ 111.70 ppm, and C-4, δ 130.86 ppm respectively. (Figure 5) The NMR spectra of various ortho, meta, pera, diortho, and dimeta, placed methoxy in benzamides entries 3, 4, 5, 11, and 12 showed singlet in the δ range 3.82–3.88 ppm and 55.34–55.90 ppm for proton and carbon resonances respectively. This assignment is done through single bond correlation of carbon and protons in HSQC NMR experiment.

Table 2: $^{13}C\{^1H\}$ NMR Chemical Shifts of *N,N*-bis(2-methoxyethyl) Substituted Benzamides for Entries 1 to 19 (1C to 7C).

Entry	1C	2C	3C	4C	5C	6C	7C
1.	136.79	126.86	128.37	129.22	128.37	126.86	172.31
2.	135.17	128.59	128.57	135.25	128.57	128.59	171.29
3.	126.35	155.04	110.95	130.18	120.80	127.92	169.82
4.	137.93	112.23	159.43	115.23	129.53	119.03	172.05
5.	132.29	128.84	113.61	160.32	113.61	128.85	172.27
6.	124.90d	157.94d	115.76d	128.87d	124.42d	130.86d	167.21
7.	132.76d	129.24d	115.32d	163.02d	115.32d	129.24d	171.40
8.	135.27	131.88	128.14	130.35	128.19	131.88	165.55
9.	139.26	125.74	135.06	129.31	135.06	125.74	169.53
10.	136.50	129.43	132.63	133.49	130.43	126.59	169.94
11.	115.03	156.45	103.95	130.24	103.95	156.45	167.27
12.	128.91	110.86	148.61	149.81	110.45	120.03	172.09
13.	114.57t	158.79dd	111.70dd	130.86t	111.70dd	158.79dd	162.18
14.	127.08dd	150.31dd	146.37dd	117.93dd	124.78dd	123.61dd	165.92dd
15.	121.22dd	158.40dd	104.17t	163.20dd	111.80dd	130.20dd	166.46
16.	126.11dd	158.49dd	117.08dd	117.39dd	153.99dd	115.72dd	165.87
17.	139.69t	110.52dd	162.70dd	104.71t	162.70dd	110.52dd	169.64t
18.	133.05	145.16	124.66	129.64	134.20	128.95	168.28
19.	143.03	128.27	123.59	147.92	123.59	128.27	170.14

d=doublet, dd=doublet of doublet, t=triplet, Other Carbon signals, where it is not mentioned, appeared as single peak.

Table 2: Continued $^{13}C\{^1H\}$ NMR Chemical Shifts of *N,N*-bis(2-methoxyethyl) for Entries 1 to 19. (8C to 13C)

Entry	8C	9C	10C	11C	12C	13C	OCH3
1.	49.68	70.63	58.90	45.41	70.97	58.90	-
2.	49.81	70.31	58.90	45.37	70.90	58.90	-
3.	49.13	71.05	58.76	45.60	70.97	58.94	55.52
4.	49.68	70.63	58.91	45.37	70.93	58.92	55.34
5.	49.89	70.64	58.94	45.53	70.90	58.96	55.34
6.	49.32	70.52	58.75	45.47	70.81	58.90	-
7.	49.77	70.22	58.84	45.29	70.80	58.86	-
8.	49.32	70.75	58.92	45.92	70.69	58.89	-
9.	49.90	69.87	58.93	45.32	70.82	59.07	-
10.	49.85	69.90	58.94	45.22	70.81	58.94	-
11.	49.13	71.07	58.77	45.97	71.30	58.89	55.79
12.	49.91	70.66	58.88	45.42	70.94	58.88	55.90
13.	49.58	70.58	58.84	45.97	70.85	58.94	-
14.	49.46	70.29	58.80	45.48	70.77	58.92	-
15.	49.56	70.28	58.88	45.45	70.80	58.83	-
16.	49.44	70.26	58.84	45.46	70.81	58.96	-
17.	49.70	69.89	58.94	45.20	70.77	58.95	-
18.	49.56	69.92	58.87	45.26	70.48	58.98	-
19.	49.70	69.62	58.86	44.98	70.69	58.89	-
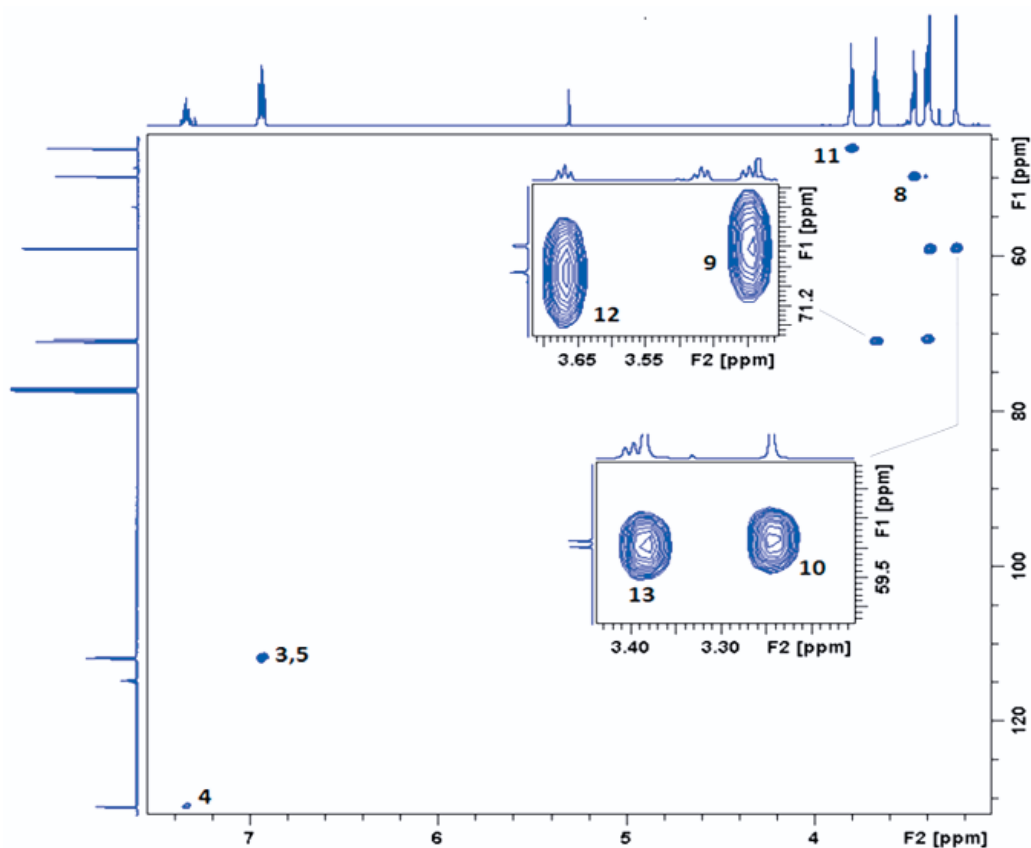
Fig. 5: 2D ^1H-$^{13}C\{^1H\}$, HSQC NMR spectrum of entry 13 in CDCl₃ showing single bond connectivity of Carbons and Protons

Mamta Sharma, Sujeet Kumar Mewar/Interesting Nuclear Magnetic Resonance studies of some N,N-bis(2-methoxyethyl) substituted Benzamides

87

Heteronuclear Multiple Bond Correlation (HMBC)

To confirm the assignments made by the use of HSQC experiment and to assign the signals corresponding to quaternary carbons, HMBC (Heteronuclear Multiple Bond Correlation) NMR spectra of benzamides were recorded (Fig. 6). This experiment established the connectivity of carbon with neighboring protons via two/three bond. In the HMBC spectra, of all benzamides H-8 protons were long range coupled to carbon signals at δ 69.62–71.07 ppm, 44.98-45.97 ppm and 162.18-172.31 ppm (Fig. 6, Scheme 2). The first resonances were assigned as C-9, second as C-11 and third were attributed to C-7 resonance. In the similar manner H-11, δ 3.63-3.82 ppm exhibited long range connectivity with C-12, δ 70.48-71.30 ppm C-8, δ 49.13-49.91 ppm and C-7. Methyl carbons of alkyl chains C-10, δ 58.75-58.94 ppm, C-13, δ 58.83-59.07 ppm, were long range coupled to the H-9, δ 3.36-3.47 ppm and H-12, δ 3.62-3.82 ppm respectively, and these in tern coupled to the respective carbons C-8 and C-11. C-1 (114.57-143.03ppm) was assigned through the long range couplings with H-5 δ 6.57-8.21 ppm and H-3 δ 6.57-8.76 ppm in benzamides where both positions C-5 and C-3 are not occupied by substitution on benzene ring. Here to demonstrate the two and three bonds important connectivity between carbon and protons, HMBC spectrum of entry 13 is shown as example in the figure 6. The over lapped signals of two Quaternary carbons C-2 and C-6 of entry 13 were assigned separately, at δ 159.46 ppm and 157.06 ppm, respectively with the help of 2D HMBC, experiment and shown their long range coupling with H-4,3 and H-4,5 of ring protons respectively. In all mono and di substituted methoxy benzamides entries 3, 4, 5, 11 and 12 the methoxy protons at δ 3.83, 3.84, 3.85, 3.82 and 3.87/3.88 ppm respectively were long range coupled to their respective quaternary ^{13}C signals at δ 155.04(C-2), 159.43(C-3), 160.31(C-4), 156.45(C-2/6) and 149.81(C-3)/148.61(C-5) ppm, respectively (Scheme 2). Beside that carbon resonances showed splitting due to the coupling with fluorine in benzamides 6, 7, and 13-17. Fluorine coupling with carbon was observed in the range 244 -251 Hz, 12-26 Hz, 4-13 Hz and 2-6 Hz for one, two, three and four bond respectively.
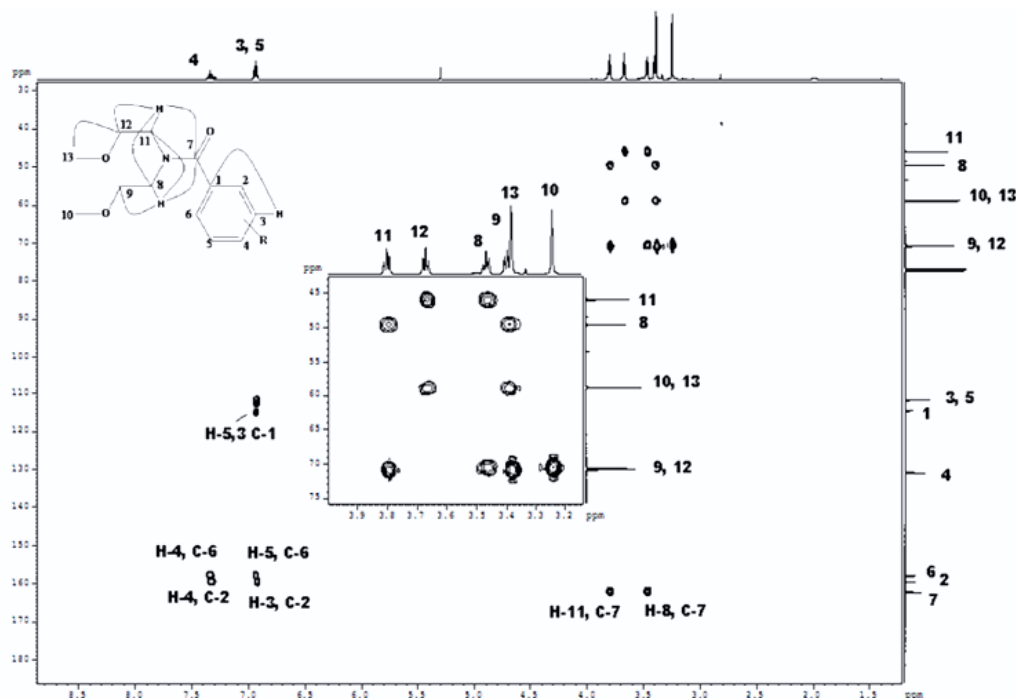

Fig. 6: 2D ^{1}H-^{13}C{^{1}H}, HMBC NMR Spectrum of Entry 13 in CDCl$_3$ showing long connectivity of Carbon with Proton

CONCLUSION

In summary, here we report the complete ^{1}H and ^{13}C NMR data of benzamide derivatives. The results obtained from the present investigation revealed that the *N,N* Dialkyl substituted benzamides are sterically crowded and floppy at room temperature, possess low barrier of rotation, cause formation of multiple rotamers. This rotation further creates broadening in the methylene proton signals. Low

temperature lowers the tumbling especially in ortho substituted benzamides and exhibited clear splitting for alkyl chain methylene proton signals. The Splitting and Sequencing of chemical shift of methylene/methyl protons at NMR Scale is affected by Substitution in benzene ring. Molecules adopted twisted geometry because of bulky alkyl chain and bulky substitution in benzene ring.

Supplementary Information (SI)

Coupling constant values of Proton and Carbon NMR, literature values of ^{1}H NMR chemical shifts of cis-trans alkyl groups, Activation energy literature values of rotamers and over lapped ^{1}H spectra of aromatic region and ^{1}H NMR showing variable temperature analysis for this article can be accessed from supplementary information.

ACKNOWLEDGMENTS

REFERENCES

1. Peterson C and Coats J 2001, Insect Repellents-Past, present and future Pestic. Outlook 12 154.

2. Cavrini F, Gaibani P, Pierro A M, Rossini G, Landini M P and Sambri V J. 2009 Chikungunya: an emerging and spreading arthropod-borne viral disease Infect Dev. Ctrie.3 10 744.

3. Monath T P, 2001 Yellow fever: an update The Lancet-Infectious Diseases1 1 11.

4. Frank C, Höhle M, Stark K and Lawrence J 2013 More reasons to dread rain on Vacation? Dengue fever in 42 German and United Kingdom Madeira tourists during autumn 2012 Eurosurveillance 1814 14.

5. Prakash S, Srivastava C P, Kumar S, Pandey K S, Kaushik M P and Rao K M 1990 N,N-Diethyl Phenyl Acetamide -A New Repellent for Periplaneta americana (Dictyoptera: Blattidae), Blattella germanica and Supella longipalpa (Dictyoptera: Blattellidae) Journal of Medical Entomology 27 6 962.

6. KalyanasundaramM and Mathew N 2006 N,N-Diethyl Phenylacetamide (DEPA): A Safe and Effective Repellent for Personal Protection Against Hematophagous Arthropods J. of Medical Entomology, 43 3 518.

7. Trigg J K 1996 Evaluation of a eucalyptus-based repellent against Anopheles spp. in Tanzania J. of the American Mosquito Control Association 12 2 243.

8. Braverman Y, Chizov-Ginzburg A and Mullens B A 1999 Mosquito repellent attracts Culicoides imicola (Diptera: Ceratopogonidae) Journal of Medical Entomology 36 1 1 13.

9. Osimitz T G and Murphy J V 1997 Neurological effects associated with use of the insect repellent N,N-diethyl-m-toluamide J. Toxicol. Clin. Toxicol. 35 435.

10. Montalbetti C A G N, and Falque V 2005 Amide bond formation and peptide coupling Tetrahedron 6110827.

11. Mack H-G and Oberhammer H 1997 Planarity of N,N-Dimethylacetamide, (CH3)2NC(O)CH3 J. Am. Chem. Soc.119 153567.

12. Troganis A N, Sicilia E, Barbarossou K, Gerothanassis, I P and Russo N 2005 Solvation Properties of N-Substituted Cis and Trans Amides Are Not Identical: Significant Enthalpy and Entropy Changes Are Revealed by the Use of variable Temperature 1H NMR in Aqueous and Chloroform Solutions and ab Initio Calculations, J. Phys. Chem. A 109 51 11878.

13. Wiberg K B, Rablen, P R, Rush, D J, Keith, T A 1995 Amides. 3. Experimental and Theoretical Studies of the Effect of the Medium on the Rotational Barriers for N,N- Dimethylformamide and N,N-Dimethylacetamide J. Am. Chem. Soc. 117 15 4261.

14. Gutowsky H S and Holm C H 1956 Rate Processes and Nuclear Magnetic Resonance Spectra. II. Hindered Internal Rotation of Amides Journal of Chemical Physics25 6 1228.

15. Mphahlele M J, Maluleka M M, Rhyman L, Ramasami P and Mampa R M 2017 Spectroscopic, DFT, and XRD Studies of Hydrogen Bonds in N-Unsubstituted 2-Aminobenzamides Molecules 22 83 1.

16. Licea R Q, Valladares J F C, Quintero A C, Padilla C R, Guerra R T, Flores R G and Waksman N 2002 NMR Detection of Isomers Arising from Restricted Rotation of the C-N Amide Bond of N-Formyl-o-toluidine and N,N'-bis-Formyl-o-tolidine Molecules 7 8 662.

17. Rabinovitz M and Pines A 1969 Hindered internal rotation and dimerization of N,N-dimethylformamide in carbon tetrachloride J. Am. Chem. Soc.91 7 1585.

18. Umemoto K and Ouchi K Hindered internal rotation and intermolecular interactions 1985Proc. Indian Acad. Sci. (Chem. Sci.)94 11.

19. Pluth M D, Bergman R G and Raymond K N 2008 Acceleration of Amide Bond Rotation by Encapsulation in the Hydrophobic Interior of a Water-Soluble Supramolecular Assembly J. Org. Chem.73 18 7132.

20. Skorupska E A, Nazarski R B, Ciechanska M, Jozwiak A, K1ys A 2013 Dynamic 1H NMR spectroscopic study of hindered internal rotation in selected N,N-dialkyl isonicotinamides: an experimental and DFT

Mamta Sharma, Sujeet Kumar Mewar/Interesting Nuclear Magnetic Resonance studies of some N,N-bis(2-methoxyethyl) substituted Benzamides

89

analysis Tetrahedron 69 38 8147.

21. Gasparro F P, and Kolodny N H 1977 NMR determination of the rotational barrier in N,N-dimethylacetamide. A physical chemistry experiment J. of Chem. Edu54 4 258.

22. Wiberg K B and Rablen P R 1995 Why Does Thioformamide Have a Larger Rotational Barrier Than Formamide ? J. Am. Chem. Soc.117 8 2201.

23. Drakenberg T, Dahlqvist K I and Forsen S 1972 Barrier to internal rotation in amides. IV. N,N-Dimethylamides. Substituent and solvent effects The Journal of Physical Chemistry 76 15 2178.

24. Kaur D, Sharma P, Bharatam P V and Dogra N, 2006 Substituent and solvent effects on the rotational barriers in selenoamides: A theoretical studyJ. Mol. Struct.(Theochem)759 1-3 41.

25. Kang Y K and Park H S 2004 Internal rotation about the C–N bond of amides J. Mol. Struct. (Theochem)676 171.

26. Duffy E M, Severance D L, and Jorgensen W L 1992 Solvent effects on the barrier to isomerization for a tertiary amide from ab initio and Monte Carlo calculations J. Am. Chem. Soc.114 19 7535.

27. Gao J 1994 Origin of the solvent effects on the barrier to amide isomerization from the combined QM/MM Monte Carlo simulations Proc. Indian Acad. Sci. (Chem. Sci.)106 2 507.

28. Fong C W and Grant H G 1980 The effect of solvents on the 13C NMR chemical shifts of the carbonyl carbon and the rotational barriers of N,N dimethylbenzamide Organic Magnetic Resonance 14 2 147.

29. Fong C W and Grant H G 1981 Solvent Effects on the Carbon - 13 NMR Chemical Shifts and Rotational Barriers of N,N-Dimethylbenzamide-Solvent Enhancedπ Polarization Z. Naturforsch 36b 585.

30. Woodbrey J C and Rogers M T 1962 Solvent Effects on the Energy Barrier for Hindered Internal Rotation in Some N,N-Disubstituted Amides J. Am. Chem. Soc.84 1 13.

31. Hammaker R M and Gugler B A 1965 An NMR study of hindered internal rotation in N,N-dialkyl amides. Journal of Molecular Spectroscopy17 2 356.

32. Pinto B M, Szarek W A and Grindley T B 1984 Effects of substitution on nitrogen on barriers to rotation of amides. 2-Evaluation of the importance of resonance effects Magnetic Resonance in Chemistry, 22 11 676.

33. Siddall III T H and Garner R H, 1966 Some Studies of Slow rotation around bonds in amides Canadian Journal of Chem. 44 20 2387.

34. Dürst T, Gryff-Keller A and Terpiński J 1983 Investigations on N,N-dialkylbenzamides by NMR spectroscopy: 5—Analysis of static and dynamic proton NMR spectra of 2-fluoro and 2,6-difluoro-N,N-dimethyl and N,N-diethyl benzamides Org. Mag. Reso. 21 11 657.

35. Keller A G and Szczecinski P 1978 Remarks on the analysis of dynamic 1H NMR spectra of A3B2 C3D2 spin systems. Internal rotation in N,N-diethylbenzamide Org. Mag. Reson. 11 5 258.

36. Gasparro F P and Kolodny N H 1977 NMR determination of the rotational barrier inN,N-dimethylacetamide. A physical chemistry experiment J. of Chem. Edu54 4 258.

37. Abraham R J, Byrne J J, Griffiths L and Perez M 2006 1H chemical shifts in NMR: Part 23, the effect of dimethyl sulphoxide versus chloroform solvent on 1H chemical shifts Magn. Reson. Chem. 44 5 491.

38. Reeves L W, Shaddick R C and Shaw K N, 1971 Nuclear Magnetic Resonance Studiesof Multi-site Chemical Exchange. III. Hindered Rotation in Dimethylacetamide, Dimethyl Trifluoro-acetamide, and Dimethyl Benzamide Canadian Journal of Chemistry 49 22 3683.

39. Reeves L W and Shaw K N, 1971 Nuclear Magnetic Resonance Studies of Multi-siteChemical Exchange. II. Hindered Rotation in N, N-Dimethyl Carbamyl FluorideCanadian Journal of Chemistry49 22 3671.

40. Bowles P, Clayden J, Helliwell M, McCarthy C, TomkinsonMandWestlundN1997Atroposelectivity in the reactions of ortholithiated aromatic tertiary amides with aldehydes J. Chem. Soc. Perkin Trans. 1 2607.

41. Clayden J, Foricher Y J Y and Lam H K 2002 Intermolecular Dearomatising Addition of Organolithium Compoundsto N-Benzoylamides of 2,2,6,6-Tetram ethylpiperidine. Eur. J. Chem. 3558.

42. Mujika J I, Gorostidi Doctoral Dissertation Dec 2005 Twisted amides: characterization of their electronic structure and analysis of their accelerated hydrolysis http://www.ehu.eus/chemistry/theory/Files/tesis_joni.pdf.

43. Lewin A H 1964 The Question of Long/Range Spin-Spin Coupling through Space: H-F Splitting over Six Bonds J. Am. Chem. Soc.86 11 2303.

44. Hu X, Zhang W, Carmichael I and Serianni A S 2010 Amide Cis−Trans Isomerizationin Aqueous Solutions of Methyl N-Formyl-d-glucosaminides and Methyl N-Acetyl-d-glucosaminides: Chemical Equilibria and Exchange Kinetics J. Am. Chem. Soc. 132 13 4641.

45. Abraham R J,Aboitiz N, Filippi M, Genesio E,Piaggio P and Sancassan F 2015Conformational analysis, part 43†. A theoretical and LIS/NMR investigation of the conformations of substituted benzamides Mag. Reson. in Chem.53 7 498.

46. Wiberg K B, Rablen P R, Rush D J and Keith T A 1995 Amides. 3. Experimental and Theoretical Studies of the Effect of the Medium on the Rotational Barriers for N, N-Dimethylformamide and N, N-Dimethylacetamide J. Am. Chem. Soc. 117 15 4261.

47. Du Ya, Hyster T D and Rovis T 2011 Rhodium (iii)-

90

Mamta Sharma, Sujeet Kumar Mewar/Interesting Nuclear Magnetic Resonance studies of some N,N-bis(2-methoxyethyl) substituted Benzamides

catalyzed oxidative carbonylation of benzamides with carbon monoxide Chem. Commun.47 12074.

48. Li M,WangC, FangP and GeH 2011Pd(II)-catalyzed decarboxylative cross-coupling of oxamic acids with potassium phenyltrifluoroborates under mild conditions Chem. Commun.47 2011 6587.

49. Harikrishna K, Balasubramaniam S, Rakshit A & Aidhen I S 2015 Some interesting 1H NMR features of ortho substituted N-methoxy-N-methyl benzamides Ind. J. of Chem. Sec. B Org. and Med. Chem.54B 77.

50. Stevenson P J 2011 Second-order NMR spectra at high field of common organic functional groups Org. Biomol. Chem. 9 2078.