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Long-Range $J(^{15}N,^{13}C)$ and $J(^{13}C,^{13}C)$ Coupling Constants via the Metal Atom in ^{13}C NMR Spectra of Square-Planar Ni(II) Complexes of the Schiff Base of (*S*)-2-(*N*-benzylprolyl)aminobenzophenone and ^{13}C-1-, ^{13}C-2- or ^{15}N-Labelled Glycine

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ABSTRACT: Chiral synthons containing either ^{13}C- or ^{15}N-labelled glycine were prepared. The ^{13}C and ^{15}N NMR spectra of the Ni(II) complex of the Schiff base of (*S*)-2-(*N*-benzylprolyl)aminobenzophenone and ^{13}C-1-, ^{13}C-2- or ^{15}N-labelled glycine were measured and assigned. The observed splitting of the carbon signals is due to long-range $J(^{13}C,^{13}C)$ and $J(^{15}N,^{13}C)$ couplings. The mutual influence of nuclei is transferred via the central Ni(II) atom in square-planar complexes. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{13}C NMR; ^{15}N NMR; long-range $J(^{13}C,^{13}C)$ and $J(^{15}N,^{13}C)$; ^{13}C- or ^{15}N-labelled glycine; Ni(II) complex; Schiff base

INTRODUCTION

^{13}C- and ^{15}N-labelled amino acids are often used in studies of peptide conformation in solutions or crystals[1] and for several biochemical purposes.[2–4] The Ni(II) complex of the Schiff base of (*S*)-2-(*N*-benzylprolyl) aminobenzophenone and glycine is a very convenient starting synthon for the asymmetric synthesis of commercially unavailable non-coded amino acids. We prepared the chiral synthons from either ^{13}C- or ^{15}N-labelled glycine. The primary aim of the preparation of these synthons was to clarify the structure of a brominated intermediate[5] of the subsequent asymmetric synthesis. During the interpretation of NMR spectra of these labelled complexes we observed an unusual long-range splitting of signals.

EXPERIMENTAL

The ^{13}C- or ^{15}N-labelled glycines (98%) were obtained from Cambridge Isotope Laboratories. Compound **1** (Fig. 1) was prepared according to a literature procedure.[6] Compounds **2**, **3** and **4** were prepared in the same way as described for the Ni(II) complex of the Schiff base from (*S*)-2-(*N*-benzylprolyl)amino-5-methylbenzophenone and glycine.[7]

Ni(II) complex of Schiff base from (*S*)-2-(*N*-benzylprolyl)aminobenzophenone and [^{15}N]glycine (**2**): yield 83%; m.p. 215–223 °C [lit.,[6] 208–212 °C (decomp.) for unlabelled compound **1**]. Calculated mass for $C_{27}H_{26}N_2^{15}NO_3Ni$, $[M + H]^+ = 499.1298$; found by high-resolution fast atom bombardment mass spectrometry (FAB-MS), $[M + H]^+ = 499.1349$.

Ni(II) complex of Schiff base from (*S*)-2-(*N*-benzylprolyl)aminobenzophenone and [^{13}C-1]glycine (**3**): yield 86%; m.p. 216–227 °C [lit.,[6] 208–212 °C (decomp.) for unlabelled compound **1**]. Calculated mass for $C_{26}\,{}^{13}CH_{26}N_3O_3Ni$, $[M + H]^+ = 499.1361$; found by high-resolution FAB-MS, $[M + H]^+ = 499.1450$.

Ni(II) complex of Schiff base from (*S*)-2-(*N*-benzylprolyl)aminobenzophenone and [^{13}C-2]glycine (**4**): yield 79%; m.p. 225–228 °C [lit.,[6] 208–212 °C (decomp) for unlabelled compound **1**]. Calculated mass for $C_{26}^{13}CH_{26}N_3O_3Ni$, $[M + H]^+ = 499.1361$; found by high-resolution FAB-MS, $[M + H]^+ = 499.1238$.

High resolution FAB-MS

FAB mass spectra were obtained with a ZAB-SEQ double-focusing mass spectrometer (VG Analytical). The fast atom beam used was generated from xenon ions, which were accelerated to 8 kV. The liquid matrix of a mixture of glycerol and thioglycerol was used for measurement. The samples were dissolved in dimethylformamide and added to the matrix. For high-resolution measurements the instrument was tuned to a resolution of 5000 (10% valley definition). The samples give $[M + H]^+$ molecular ions in the spectra.

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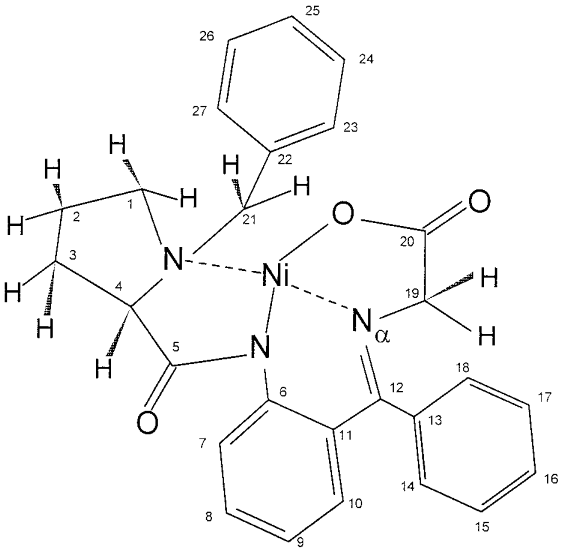

1: natural abundance

2: $N_\alpha = {}^{15}N$ (98%)

3: $C19 = {}^{13}C$ (98%)

4: $C20 = {}^{13}C$ (98%)

Figure 1. Structures of compounds **1** (^{13}C and ^{15}N in natural abundance) **2**[$N_\alpha = {}^{15}N$ (98%)], **3** [C-19 = ^{13}C (98%)] and **4** [C-20 = ^{13}C (98%)].

NMR measurements

The ^{13}C NMR spectra were obtained in $CDCl_3$ solutions using a Bruker AMX-360 spectrometer equipped with a multinuclear 5 mm tunable probe at 90.56 MHz. ^{13}C chemical shifts are given with respect to the solvent signal ($\delta^{13}C = 77.00$ ppm). The concentration of compounds for measurement of ^{13}C NMR spectra was 20 mg in 0.5 ml of $CDCl_3$. The conditions for ^{13}C NMR measurements were spectral width SW = 15 625 Hz, pulse width $P_1 = 5$ μs (60° flip angle), number of data points after Fourier transformation TD-64K, relaxation delay $D_1 = 2$ s, number of transients NS = 1024 and digital resolution = 0.24 Hz per point.

The ^{15}N NMR spectra were obtained in $CDCl_3$ solutions with the same instrument at 36.49 MHz. ^{15}N chemical shifts are given with respect to external CH_3 $^{15}NO_2$($\delta^{15}N = 0.00$ ppm). For measurement of ^{15}N NMR spectra, the concentration of **3** and **4** was 130 mg in 0.5 mg of $CDCl_3$ and that of **2** was 50 mg in 0.5 ml of $CDCl_3$. The conditions for ^{15}N NMR measurement of **3** with inverse gated decoupling were as follows: SW = 7814 Hz, $P_1 = 6$ μs (45° flip angle), number of data points after Fourier transformation TD = 32K, acquisition time AQ = 2.1 s, $D_1 = 7$ s, NS = 34 700 digital resolution = 0.24 Hz per point. ^{15}N NMR spectra of **2**, **3** and **4** were measured by refocused INEPT arranged to $J = 3$ Hz,[8] SW = 11 111 Hz, $P_1 =$ 12 μs, $P_2 = 24$ μs, AQ = 1.47 s, number of data points after Fourier transformation TD = 32K, $D_1 = 1.5$ s, NS = 17 000 and digital resolution = 0.34 Hz per point.

The $\Delta^{15}N(^{13}C)$ and $^nJ(^{15}N,^{13}C)$ values were obtained from the ^{15}N NMR spectrum of **2** measured by refocused INEPT and optimized to the following parameters: SW = 7353 Hz, $P_1 = 12$ μs, $P_2 = 24$ μs, AQ = 4.45 s, $D_1 = 2$ s, $D_3 = D_4 = 54$ ms and NS = 9432, and processed by Gaussian multiplication with parameters LB = −0.2 Hz and GB = 0.3 to 128K points (digital resolution 0.05 Hz per point.

The ^{15}N NMR spectrum of **2** was also measured with a multinuclear 10 mm probe at a concentration of 500 mg in 2 ml of $CDCl_3$ by inverse gated decoupling with $P_1 = 10$ μs (45°), NS = 9000 and other parameters the same as for **3**.

X-ray analysis

A single red crystal of **2** was grown by crystallization from benzene. The crystal was cut to a size 0.3 × 0.2 × 0.2 mm and used for data collection by a CAD4-MACHIII-PC diffractometer with graphite-monochromated Mo Kα radiation. Lattice characteristics were determined from a least-squares fit of 25 reflections in the range of 13–15°. The crystal is orthorhombic, *P*212121, with the parameters $a = 9.0459$, $b = 9.7219$, $c = 26.455$.

Intensity data were collected using $\theta - 2\theta$ scans in the interval $0 \leqslant 2\theta \leqslant 54°$ ($h - 10$ to 11, $k - 11$ to 12, $l - 30$ to 33). The absorption and extinction correction was applied. Three standard reflections monitored every hour showed some random variation in intensity, maximally about 2.4%. A total of 4182 reflections were collected and were reduced to 3967 unique reflections [$R_{(\text{int})} = 0.022$ and $R_{(\sigma)} = 0.023$]. The structure was solved by direct methods using the program SHELX-86. Refinement was carried out by full-matrix least squares of *F* SHELX-93. Positions of all hydrogen atoms were found from the difference Fourier map. The *B* factors of non-hydrogen atoms were refined anisotropically and those of hydrogen atoms were refined isotropically. The structure was refined to $R = 0.039$ and $wR = 0.087$. A final difference Fourier synthesis showed $\Delta\rho_{(\min)} = -0.27$ e Å^{-3} and $\Delta\rho_{(\max)} = 0.37$ e Å^{-3}.

RESULTS AND DISCUSSION

The ^{13}C NMR chemical shifts of compounds **1**–**4** (Fig. 1) are given in Table 1. The assignments were made by comparison with recently published NMR spectra of similar complexes.[7,8] There exists a slow (with regard to the NMR time-scale) rotation of a phenyl ring around the C-12—C-13 bond in complexes **1**–**4**. The speed of

Table 1. ^{13}C NMR chemical shifts and coupling $J(^{15}N,^{13}C)$ and $J(^{13}C,^{13}C)$ of compounds 1–4 in $CDCl_3$ at 23 °C

	1	2		3		4	
Carbon	$\delta^{13}C$ (ppm)	$\delta^{13}C$ (ppm)	$^nJ(^{15}N_\alpha, ^{13}C)$ (Hz)	$\delta^{13}C$ (ppm)	$^nJ(^{13}C19, ^{13}C)$ (Hz)	$\delta^{13}C$ (ppm)	$^nJ(^{13}C20, ^{13}C)$ (Hz)
1	57.42	57.43		57.43		57.43	
2	23.63	23.64		23.64		23.64	
3	30.65	30.66		30.66		30.67	
4	69.80	69.81	2.9	69.82	4.4	69.82	2.8
5	181.30	181.31		181.31		181.32	
6	142.46	142.47	1.1	142.49		142.48	
7	124.19	124.20		124.21		124.20	
8	132.15	132.16		132.17		132.16	
9	120.78	120.79		120.79		120.79	
10	133.11	133.13	2.3	133.13		133.12	
11	125.10	125.11	2.9	125.12	3.3	125.11	
12	171.56	171.55	12.5	171.53	0.9	171.56	5.0
13	134.55	134.56	1.2	134.57	3.7	134.56	
14	126.19	126.20		126.21		126.20	
15	129.28	129.29		129.29		129.28	
16	129.67	129.69		129.69		129.68	
17	129.54	129.55		129.55		129.54	
18	125.60	125.62		125.62		125.62	
19	61.20	61.22	4.8	61.24		61.21	56.8
20	177.24	177.24	3.6	177.28	56.8	177.24	
21	63.05	63.06	2.5	63.06	3.5	63.06	2.7
22	133.24	133.25		133.26		133.25	
23	131.66	131.68		131.68		131.67	
24	128.86	128.87		128.87		128.87	
25	129.05	129.07		129.07		129.07	
26	128.86	128.87		128.87		128.87	
27	131.66	131.68		131.68		131.67	

this rotation has been studied previously[8] on similar compounds by the 2D-NOESY technique. Complexes with bulky substituents (dimethylamino) on carbon C-19 do not exhibit such rotation and signals of their C-14 to C-18 carbons could be assigned on the basis of NOESY interactions of H-14 and H-18 protons with other parts of molecule. Carbons C-14 and C-15 are situated above the plane of the complex in the case of frozen rotation. The assignment of C-14 to C-18 in **1**–**4** has been made by comparison with recently published[8] NMR spectra of both C-19 dimethylamino derivatives of complex **1**.

$J(^{15}N,^{13}C)$

The largest coupling $J(^{15}N,^{13}C)$ was observed on C-12 in **2** (12.5 Hz). This result is not surprising because the interaction is transferred by the shortest sp^2–sp^2 bond. The second one-bond coupling $^1J(^{15}N,^{13}C\text{-}19)$ is more than twice as small (4.8 Hz). The stereospecificity of $^nJ(^{15}N,^{13}C)$ was observed in cases when $n = 2$ and 3 in the neighbourhood of the sp^2-hybridized $^{15}N_\alpha$ nitrogen. The carbons located *trans* from the point of view of the $^{15}N_\alpha$—Ni bond have smaller or no $^nJ(^{15}N,^{13}C)$. Thus $^2J(^{15}N,^{13}C\text{-}11)$ is larger than $^2J(^{15}N,^{13}C\text{-}13)$. The couplings $^3J(^{15}N,^{13}C\text{-}6)$ and $^3J(^{15}N,^{13}C\text{-}10)$ were found to be 1.1 and 2.3 Hz whereas no couplings with ^{15}N were observed on C-14 and C-18. This may be due to either the stereospecificity of $^3J(^{15}N,^{13}C)$ or the presence of the freely rotating phenyl group around the C-12–C-13 bond. This rotation has already been observed in similar complexes.[8] In our case this rotation is not so fast as to cause the coalescence or erasure of the C-14 and C-18 or C-15 and C-17 signals, but the small couplings of *ca.* 1 Hz could be mashed by broadening of these signals. The $^nJ(^{15}N,^{13}C)$ couplings observed in the benzylproline part of **2** cannot be explained by long-range couplings through six or eight C—C and C—N bonds, which should be undetectable. The mechanism of transfer must be different.

Through-space homonuclear long-range couplings have recently been described by Schröder Haslinger[9] and through-space heteronuclear long-range couplings between carbon and fluorine by Lyga *et al.*[10] To our knowledge, there are no reports of heteronuclear through-space long-range interactions between carbon and nitrogen. Through-space long-range couplings are usually explained by overlapping of atomic orbitals.[9,10] Through-space coupling via lone-pair orbitals of an oxygen[11] or a sulphur[12] atom has also been observed. One of the probable explanations of long-range interactions observed by us is a transfer through the Ni orbitals above the plane of complex. X-ray analysis of **2** has shown that the N—Ni bonds lie in the same plane (see Fig. 2). Full X-ray crystallographic data have been published elsewhere.[13] Carbon C-21 and protons H-21 and H-4 are situated above the plane of the complex. Orbitals of the above-mentioned nuclei can be in contact with or overlap the Ni orbitals. The central Ni^{2+} atom should have the electronic configuration $(e_g)^4(b_{2g})^2(a_{1g})^2(b_{1g})^0$ because the complex shows diamagnetic properties and has a square-like arrangement of a coordination polyhedron as shown on Fig. 2. The doubly degenerate orbital e_g is lower from the energy point of view. The orbital e_g corresponds to the original d_{xz} and d_{yz} orbitals, and the orbital b_{2g} corresponds to the original d_{z^2} orbital. The vacant b_{1g} ($d_{x^2-y^2}$) participates in the hybridization to form d_{sp^2} orbitals. These orbitals are acceptors of electron pairs from ligand donor atoms. These Ni orbitals can transfer the couplings from the glycine to the benzylproline part of molecule. No couplings were observed involving nitrogens with chemical shifts of −270.76 and −349.78 ppm in **3** and **4** and only small couplings of 0.5 and 2.6 Hz were observed with the same nitrogen signals in **2** (see later), which means that couplings are badly transferred in the plane of the complex. No coupling with labelled nuclei was observed on benzylic protons or the H-4 proton in spite of their σ-bonding orbitals being in contact with Ni orbitals above the plane of complex. If the interactions are transferred by orbitals above the plane of the complex they must be transferred only in a single direction through the σ-bonds C-21—H-21 and C-4—H-4 towards the heavier atom (carbon). The other explanation of 'long-range' transfer is that the couplings are transferred in the plane of the complex via N_α—Ni—N bonds. The poor transfer in the plane of the complex noted above can be explained by a usually small value of $^2J(^{15}N,^{15}N)$ in the range 1–8.5 Hz.[14]

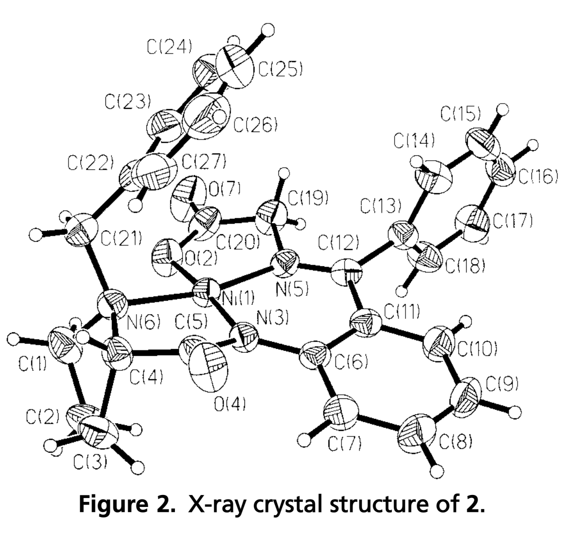

Figure 2. X-ray crystal structure of **2**.

$J(^{13}C,^{13}C)$

A similar type of long-range spin–spin interaction observed in ^{15}N-labelled **2** was observed also for ^{13}C-labelled **3** and **4**. The long-range couplings $J(^{13}C,^{13}C)$ were observed in the benzylproline part of the complex on carbons C-4 and C-21 in **3** and **4**. In this case, the transfer of this interaction via Ni orbitals in the plane of complex or above the plane also occurs, since the distance of coupled nuclei is too long through the normal σ-bonds (seven and nine bonds in the **3** and eight and ten bonds in the **4**). Such a type of long-range interactions has not been reported previously.

$\delta^{15}N$

The ^{15}N NMR spectra of **2**, **3** and **4** were measured by refocused INEPT arranged for $J = 3$ Hz. The same value of the coupling constant was successfully used[8] for ^{15}N NMR INEPT measuring some substituted Ni(II) complexes of the Schiff base from (*S*)-2-(*N*-benzylprolyl)aminobenzophenone and substituted glycine. None of the three measured compounds gave all three signals of nitrogens with this type of measurement. Only signals with chemical shifts of −195.11 ppm (imine-type nitrogen) and − 270.79 ppm (amide-type nitrogen) in **2** were observed. Signals at −195.48 ppm with coupling $^1J(^{15}N,^{13}C) = 4.7$ Hz and a singlet at −270.76 ppm were observed for **3**. Signals at −195.07 ppm with coupling $^2J(^{15}N,^{13}C) = 3.5$ Hz and a singlet at −270.76 ppm were observed for **4**. Compound **3** was also measured by the inverse gated decoupling method and gave three signals. The third signal belongs to a proline-type nitrogen with a chemical shift of −349.78 ppm.

$J(^{15}N,^{15}N)$

X-ray data do not support our theory of C-4 positioned above the plane of the complex. In solution, of course, the conformation of the complex can be different. We tried to confirm if the couplings are also transferred in the plane of complex. We have detected small $J(^{15}N,^{15}N)$ couplings at both unlabelled nitrogens of **2**: 0.5 Hz at ^{15}N with a chemical shift of −270.76 ppm and 2.6 Hz at ^{15}N with a chemical shift of −349.78 ppm. For these measurements it was necessary to use the 10 mm probe with a high concentration of compound. For example, the signal-to-noise ratio was 10 710 for the N_α nitrogen, 23 for the amide-type nitrogen ($\delta = -270.76$ ppm) and only 6 for the proline-type nitrogen ($\delta = -349.78$ ppm).

$\Delta^{15}N(^{13}C)$

Isotopic substitution may lead to changes in the nuclear shielding in the ^{15}N NMR spectra of **2**. We observed ^{13}C satellites of the $^{15}N_\alpha$ NMR signal of **2** corresponding to the couplings determined in the ^{13}C NMR spectrum. We calculated the intrinsic isotope effect of ^{13}C on the $^{15}N_\alpha$ nitrogen in accordance with Gombler's nomenclature[15] as

$$^{n}\Delta^{15}N(^{13}C) = \delta^{15}N(^{12}C) - \delta^{15}N(^{13}C).$$

We calculated four primary intrinsic isotope effects: $^{1}\Delta^{15}N_\alpha(^{13}C\text{-}12) = +0.0589$ ppm, $^{1}\Delta^{15}N_\alpha(^{13}C\text{-}19) = +0.0258$ ppm, $^{2}\Delta^{15}N_\alpha(^{13}C\text{-}20) = +0.0011$ ppm and $^{2}\Delta^{15}N_\alpha(^{13}C\text{-}11) = -0.0005$ ppm. The last two values are under the resolution limit of 0.0015 ppm per point and are insignificant. The one-bond intrinsic isotope effect is largest for C-12. This result corresponds to the largest $^{1}J(^{15}N,^{13}C)$ coupling.

CONCLUSION

Stereospecificity of $^{2}J(^{15}N,^{13}C)$ was observed in the neighbourhood of the sp^2-hybridized $^{15}N_\alpha$ nitrogen in the Ni(II) complex of the Schiff base from (*S*)-2-(*N*-benzylprolyl)aminobenzophenone and [^{15}N]glycine (**2**). The couplings $^{n}J(^{15}N_\alpha,^{13}C)$ observed in the benzylproline part of the complex on C-4 and C-21 in **2** and the long-range couplings $^{n}J(^{13}C,^{13}C)$ observed in the benzylproline part of the complex on C-4 and C-21 in **3** and **4** cannot be transferred by C—C or C—N bonds owing to the large distance involved. Transfer of this spin–spin interaction occurs through the Ni atom via orbitals in the plane or above the plane this planar complex. The small splitting of the proline-type nitrogen signal and amide-type nitrogen signal [$J(^{15}N,^{15}N)$] was observed only in the $^{15}N_\alpha$-labelled complex **2**.

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