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Long-range heteronuclear coupling constants in 2,6-disubstituted purine derivatives

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Four- and five-bond heteronuclear *J*-couplings between the hydrogen H-8 and carbons C-6 and C-2 in a series of 7- and 9-benzyl substituted purine derivaties with variuous substituents in positions 2 and 6 were studied by coupled ^{13}C NMR and H,C-HMBC experiments and by DFT calculations. We have found that for some of the derivatives, the five-bond coupling H8-C2 is higher than the four-bond H8-C6 coupling, which is also evidenced by a stronger crosspeak in the HMBC. This finding contradicts the generally accepted opinion that only strong three-bond crosspeaks and one weak four-bond H8-C6 crosspeak can be observed in the HMBC spectra of purine derivatives. The misinterpretation of HMBC spectra may lead to an incorrect determination of the purine derivatives' structure. Copyright © 2012 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: NMR; ^{1}H; ^{13}C; long-range coupling; purine derivatives; DFT calculations

Introduction

Naturally occurring purines are the basic constituents of nucleic acids; they also interact with enzymes and other proteins as components of the cofactors and signal molecules.[1] Adenosine 5′-triphosphate (ATP) controls the energy metabolism of cells, and nicotinamide adenine dinucleotide (NAD+, NADH) and flavin adenine dinucleotide (FAD, $FADH_2$) are the key cofactors not only of the cellular citric acid cycle, which is involved in the cellular oxidation/reduction processes.[2] Another molecule of biological relevance is acetyl-coenzyme A, which is of central importance for metabolism.

Purine derivatives bearing diverse types of substituents display a broad spectrum of biological activities,[3] including an interferon-inducing effect or more often an inhibitory effect against leukotriene A_4 hydrolase, sulfotransferase, phosphodiesterase, kinase, and other enzymes.[4] Modified nucleosides and nucleotides are very important classes of compounds used in the therapy of a wide variety of diseases, since they can act as antiviral,[5–8] antitumor[9,10] or antimicrobial[11] agents.

The distribution of electrons around the purine skeleton affects not only its chemical properties and reactivity but also the NMR parameters. The nature of the substituent is reflected in the NMR chemical shifts and nuclear spin–spin coupling constants, which makes NMR spectroscopy an excellent tool for investigating and interpreting the structure, reactivity and intermolecular interactions in terms of the electron distribution.[12] The ^{13}C and ^{15}N NMR chemical shifts as well as ^{1}H–X coupling constants can be used not only to distinguish between different regioisomers,[13] but also to reflect equally well the positions of protons, which enables the study of the tautomeric equilibria.[2,14–20]

The assignment of the carbon signals is usually done using three-bond H-C heteronuclear *J*-coupling.[21] A schematic graphical representation of the 'building blocks' for the assignment of purine derivatives signals with C-6 substituents is given in Fig. 1. It is generally believed that, for derivatives substituted at both positions 2 and 6, small four-bond interactions H8-C6 can be used for the assignment of the carbon signals. The H8-C6 coupling can be identified in the HMBC spectra as a crosspeak with lower intensity.[22]

In this paper, we present a combined experimental and computational study of the four- and five-bond heteronuclear couplings H8-C6 and H8-C2. The experimentally studied compounds are depicted in Fig. 2. We demonstrate that the five-bond coupling is of a comparable magnitude with the four-bond coupling and a structure determination based on the HMBC patterns can lead to incorrect structures and/or the incorrect assignment of signals.

Experiment

The syntheses of compounds **1–4** and **6–7** have been described previously.[23–25] For the selective preparation of 7-substituted purines, an alternative synthetic approach could be used.[26] The preparation of compounds **5**, **9** and **10** will be described in a separate paper with the description of their biological activities. Briefly, compound **5** was obtained after hydrolysis of compound **1**; compound **9** was prepared by methanolysis of compound **6**, and compounds **8** and **10** were prepared by microwave-assisted reactions of compound **6** with 1 M hydrochloric acid (compound **8**) or with dibutylamine (compound **10**).

The NMR spectra were measured on a Bruker Avance 600 (with ^{1}H at 600.13 MHz and ^{13}C at a frequency of 150.92 MHz) and/or Bruker Avance 500 (with ^{1}H at 499.95 MHz and ^{13}C at 125.71 MHz) using a 5 mm TXI cryoprobe and about 5–10 mg of sample in 0.6 ml of $CDCl_3$ or DMSO-d_6. The chemical shifts are given in δ-scale (with the ^{1}H and ^{13}C referenced to TMS or to

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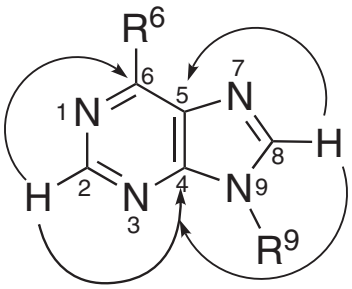
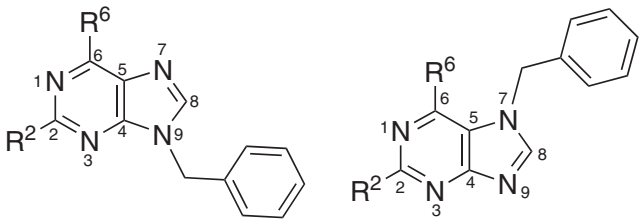


Figure 1. Schematic representation of the 'HMBC building blocks' (three-bond coupling pathways) for the assignment of purine derivatives signals with C-6 substituents.



	R^2	R^6
1	NH_2	Cl
2	Cl	Cl
3	H	Cl
4	NH_2	$NHCH_3$
5	NH_2	OH

	R^2	R^6
6	Cl	Cl
7	NH_2	Cl
8	OH	OH
9	Cl	OCH_3
10	Cl	NBu_2

Figure 2. The studied compounds and their numbering.

DMSO using δ(DMSO) 2.50 and 39.7 ppm, respectively). The typical experimental conditions for the ^{1}H NMR spectra were 32 scans, a spectral width of 6 kHz, and an acquisition time of 5 s, yielding 60 K data points. The FIDs were zero-filled to 128 K data points. The coupled ^{13}C NMR spectra were acquired using a gated decoupling pulse sequence (decoupling during d1). The ^{13}C spectra were also acquired with selective decoupling of H-8. The coupled ^{13}C and selectively decoupled ^{13}C experiments (30 mg of a sample dissolved in 0.6 ml of a solvent) were acquired with a spectral width of 70 ppm, offset of 135 ppm, d1 = 3 s, acquisition time of 3.7 s yielding 64 K data points, which were zero-filled to 128 K data points. The 2D-homonuclear (H,H-COSY) and 2D-heteronuclear (H,C-HSQC and H,C-HMBC) experiments were performed for the structural assignments of the ^{1}H and ^{13}C signals (using standard 2D-NMR pulse sequences of Bruker software).

The geometry optimizations and NMR parameters calculations were conducted using the Gaussian 09 software package.[27] All of the structures were optimized at the DFT level of theory using the B3LYP functional.[28,29] For geometry optimization, the standard 6-31 + G(d,p) basis set was used, and for the shielding and coupling constants, a slightly larger 6-311++G(d,p) basis set was utilized. The vibrational frequencies and free energies were calculated for all of the optimized structures, and the stationary-point character (a minimum) was thus confirmed. The optimizations and shielding- and coupling-constant calculations were done in vacuum. We have previously explored various approaches for modeling the solvent effects on the calculation of NMR parameters. We have shown that a large number of molecular dynamics snapshot geometries have to be averaged to model the solvent effects reasonably and that implicit solvent models (e.g. PCM) fail in the calculation of the solvent effects on NMR parameters.[30,31] Further discrepancy between the calculated and experimental data can be caused by the inaccuracy of the DFT calculations and neglecting vibrational averaging.[32]

The NMR spectra signal assignment (C-1′ - C-4′ correspond to C-*ipso*, C-*ortho*, C-*meta* and C-*para* of the benzyl substituent) is given as follows:

9-Benzyl-6-chloro-9*H*-purin-2-amine (**1**): ^{13}C NMR (150.9 MHz, DMSO): δ = 46.3 (CH_2); 123.5 (C-5); 127.4 (C-2′), 128.0 (C-4′), 129.0 (C-3′); 136.8 (C-1′); 143.5 (C-8); 149.7 (C-6); 154.3 (C-4); 160.1 (C-2) ppm. ^{1}H NMR (600.1 MHz, DMSO): δ = 5.29 (s, 2H, CH_2); 6.92 (bs, 2H, NH_2); 7.26 – 7.38 (m, 5H, H-2′, H-3′, H-4′), 8.22 (s, H-8) ppm.

9-Benzyl-2,6-dichloro-9*H*-purine (**2**): ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 48.0 (CH_2); 128.0 (C-2′), 129.0 (C-4′), 129.3 (C-3′); 130.6 (C-5); 133.9 (C-1′); 145.5 (C-8); 151.8 (C-6); 153.1 (C-4 and C-2) ppm. ^{1}H NMR (499.9 MHz, $CDCl_3$): δ = 5.42 (s, 2H, CH_2); 7.32 (m, 2H, H-2′); 7.36 – 7.41 (m, 3H, H-3′, H-4′), 8.07 (s, H-8) ppm.

9-Benzyl-6-chloro-9*H*-purine (**3**)^{13}C NMR (125.7 MHz, DMSO): δ = 47.3 (CH_2); 127.9 (C-2′), 128.3 (C-4′), 129.0 (C-3′); 131.0 (C-5); 136.2 (C-1′); 147.7 (C-8); 149.4 (C-6); 151.9 (C-2); 152.0 (C-4) ppm. ^{1}H NMR (499.9 MHz, DMSO): δ = 5.42 (s, 2H, CH_2); 7.32 (m, 2H, H-2′); 7.36 – 7.41 (m, 3H, H-3′, H-4′), 8.07 (s, H-8) ppm.

9-Benzyl-N^6-methyl-9*H*-purin-2,6-diamine (**4**): ^{13}C NMR (125.7 MHz, DMSO): δ = 27.2 (CH_3); 45.6 (CH_2); 113.6 (C-5); 127.2 (C-2′), 127.6 (C-4′), 128.8 (C-3′); 137.3 (C-8); 137.9 (C-1′); 151.0 (C-4); 155.6 (C-6); 160.6 (C-2) ppm. ^{1}H NMR (499.9 MHz, DMSO): δ = 5.42 (s, 2H, CH_2); 7.32 (m, 2H, H-2′); 7.36 – 7.41 (m, 3H, H-3′, H-4′), 8.07 (s, H-8) ppm.

9-Benzylguanine (**5**): ^{13}C NMR (150.9 MHz, DMSO): δ = 47.8 (CH_2); 110.4 (C-5); 128.2 (C-2′), 128.8 (C-4′), 129.5 (C-3′); 135.8 (C-1′); 137.9 (C-8); 150.7 (C-4); 155.0 (C-6); 155.7 (C-2) ppm. ^{1}H NMR (600.1 MHz, DMSO): δ = 5.30 (s, 2H, CH_2); 7.05 (bs, 2H, NH_2); 7.28 – 7.40 (m, 5H, H-2′, H-3′, H-4′), 8.87 (s, H-8) ppm.

7-Benzyl-2,6-dichloro-7*H*-purine (**6**): ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 50.9 (CH_2); 121.7 (C-5); 127.0 (C-2′), 129.0 (C-4′), 129.4 (C-3′); 134.1 (C-1′); 143.9 (C-6); 150.4 (C-8); 153.2 (C-2); 163.5 (C-4) ppm. ^{1}H NMR (499.9 MHz, $CDCl_3$): δ = 5.69 (s, 2H, CH_2); 7.18 (m, 2H, H-2′); 7.36 – 7.42 (m, 3H, H-3′, H-4′), 8.27 (s, H-8) ppm.

7-Benzyl-6-chloro-7*H*-purin-2-amine (**7**): ^{13}C NMR (125.7 MHz, DMSO): δ = 49.4 (CH_2); 115.0 (C-5); 126.6 (C-2′), 128.0 (C-4′), 129.0 (C-3′); 137.4 (C-1′); 142.6 (C-6); 150.2 (C-8); 160.3 (C-2); 164.6 (C-4) ppm. ^{1}H NMR (499.9 MHz, DMSO): δ = 5.56 (s, 2H, CH_2); 6.64 (bs, 2H, NH_2); 7.13 (m, 2H, H-2′); 7.27 -7.36 (m, 3H, H-3′, H-4′), 8.55 (s, H-8) ppm.

7-Benzylxanthine (**8**): ^{13}C NMR (125.7 MHz, DMSO): δ = 49.0 (CH_2); 106.2 (C-5); 127.7 (C-2′), 128.1 (C-4′), 128.8 (C-3′); 137.3 (C-1′); 142.8 (C-8); 149.7 (C-4); 151.4 (C-2); 155.7 (C-6) ppm. ^{1}H NMR (499.9 MHz, DMSO): δ = 5.41 (s, 2H, CH_2); 7.27 – 7.36 (m, 5H, H-2′, H-3′, H-4′), 8.13 (s, H-8); 10.87 (bs, 1H) and 11.58 (bs, 1H, H-1 and H-3) ppm.

7-Benzyl-2-chloro-6-methoxy-7*H*-purin (**9**): ^{13}C NMR (125.7 MHz, DMSO): δ = 50.2 (CH_2); 55.1 (CH_3); 111.8 (C-5); 127.5 (C-2′), 128.2 (C-4′), 128.9 (C-3′); 136.8 (C-1′); 148.5 (C-8); 151.3 (C-2); 157.5 (C-6); 162.8 (C-4) ppm. ^{1}H NMR (499.9 MHz, DMSO): δ = 4.05 (s, 3H, CH_3); 5.54 (s, 2H, CH_2); 7.27 – 7.31 (m, 5H, H-2′, H-4′), 7.35 (m, 2H, H-3′); 8.73 (s, H-8) ppm.

7-Benzyl-*N*,*N*-dibutyl-2-chloro-7*H*-purin-6-amine (**10**): ^{13}C NMR (125.7 MHz, DMSO): δ = 13.8 (C-4″); 19.5 (C-3″); 29.1 (C-2″); 49.5 (C-1″); 50.9 (CH_2); 113.8 (C-5); 126.3 (C-2′), 128.0 (C-4′), 128.9 (C-3′); 137.0 (C-1′); 149.9 (C-8); 151.9 (C-2); 155.6 (C-6); 163.6 (C-4) ppm.

^{1}H NMR (499.9 MHz, DMSO): $\delta = 0.75$ (t, 6H, $J_{4'',3''} = 7.3$, H-4″); 1.01 (m, 4H, H-3″); 1.35 (m, 4H, H-2″); 3.39 (m, 4H, H-1″); 5.56 (s, 2H, CH_2); 6.97 (m, 2H, H-2′); 7.24 – 7.32 (m, 3H, H-3′, H-4′), 8.62 (s, H-8) ppm.

Results and Discussion

In a typical HMBC spectrum of 2,6-disubstituted purine derivatives (refer to Fig. 3), we can observe strong three-bond crosspeaks between the hydrogen H-8 and carbons C-4 and C-5. The strong crosspeaks are accompanied by one or two weak crosspeaks corresponding to H8-C6 and/or H8-C2. The stronger crosspeak from these two low-intensity crosspeaks is believed to be the H8-C6 correlation.[22] However, we have observed that for many purine derivatives, the long-range crosspeaks are of a comparable magnitude, and in some cases, the magnitude of the H8-C2 crosspeak is larger than that of the H8-C6 crosspeak. The intensity of a HMBC crosspeak is dependent on the match between heteronuclear coupling constant and the experimental delay of the anti-phase evolution (1/2 J). For heteronuclear couplings lower than 1 Hz, the evolution delay should be larger than 500 ms, which leads to a strong signal suppression by relaxation. We performed optimization of the evolution delay and we have observed that for longer delays, the gain of intensity of the weak crosspeaks was overridden by the relaxation losses. Therefore, all the HMBC spectra reported in this paper were measured with evolution delay of 50 ms. Using this setup, the intensity of the long-range HMBC crosspeaks is mainly governed by the value of the *J*-coupling. The HMBC spectra of all of the studied compounds are shown in Supporting information.

The long-range heteronuclear coupling can be observed in hydrogen-coupled ^{13}C spectra. Because of the small values of the four- and five-bond coupling constants, line narrowing and longer acquisition times are necessary, which makes this technique possible only for concentrated samples or ^{13}C labeled compounds. We measured the coupled ^{13}C spectra of compounds **2**, **5** and **6**. A part of the coupled spectrum of compound **5** depicting the signals of C-2 and C-6 is shown in Figure S11 in the SI. For comparison, the spectrum obtained with a selective decoupling of H-8 is also shown in the figure. The experimental values of the long-range coupling constants obtained from the coupled ^{13}C spectra are discussed below. Given the experimental difficulties in obtaining the coupled spectra, we estimated qualitatively the couplings and their relative intensities from the HMBC spectra.

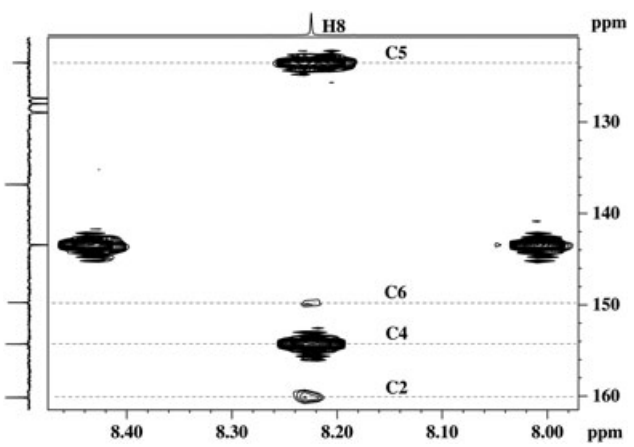


Figure 3. The H-8 region of the HMBC spectrum of compound **1**.

The uncertainty in the signal assignment based on the HMBC patterns may lead to incorrect structure determination. For some 7-substituted purine derivatives, the spatial proximity obtained from the NOE experiments can be used to determine the structure unequivocally. This approach is demonstrated on compound **10**. In the ROESY spectrum of compound **10**, we observed crosspeaks between the benzyl part of the molecule and the dibutylamino part, which is possible only for the 7-benzyl-6-dibutylamino derivative. In some cases, the assignment of the carbon signal could be obtained from the HMBC spectra observing the crosspeaks of the NH_2, $HNCH_3$ or OCH_3 groups. For some derivatives, the spectral assignment could be done only with the help of calculated chemical shifts.

We performed geometry optimization and shielding- and coupling-constant calculations for a series of purine derivatives with various substituents in positions 2 and 6 and a methyl substituent in position 7 or 9. We believe that the change of the benzyl substituent to a methyl group can cause only minor differences in the calculated coupling constants, while the calculations are much less demanding. We confirmed this for compound **6**. The differences in the calculated H8-C2 and H8-C6 coupling constants between compound **6** (7-benzylderivative) and 2,6-dichloro-7-methyl-7*H*-purine were lower than 0.01 Hz.

Both the calculated *J*(H8-C2) and *J*(H8-C6) were always positive. Interestingly, the calculated values of *J*(H2-C8) were always negative, and the absolute values were close to 0.2 Hz, which is in agreement with the lack of crosspeaks between H-2 and C-8 in the HMBC spectra of the purine derivatives. For all derivatives with the same substituents in both positions 2 and 6 except for the dihydroxyderivatives, the calculated chemical shift of C-6 was lower than that of C-2 by 1–6 ppm for 9-methyl- and by 11–19 ppm for the 7-methylderivatives.

In the HMBC spectrum of compound **1** (Fig. 3), we observed weak crosspeaks to both C-2 and C-6 with the C-2 crosspeak being slightly more intensive. This observation is in agreement with the calculated coupling constants (Table 1, Entry 8), where the H8-C2 coupling was 0.61 Hz compared to 0.32 Hz of the H8-C6 coupling. The signals of the carbon atoms C2 and C4 are unfortunately overlapped in the spectrum of compound **2**, and therefore, the H8-C2 crosspeak (if present) is overlapped by the strong H8-C4 crosspeak in the HMBC spectrum. We measured the coupled ^{13}C NMR spectrum and ascertained that the experimental long-range couplings 0.4 and 0.3 Hz were very close to the calculated values (0.52 and 0.19 Hz, Table 1, Entry 4). In agreement with the small value of the H8-C6 coupling constant, no H8-C6 crosspeak was observed in the HMBC spectrum. Similarly, in the HMBC spectrum of compound **3**, we did not observe any H8-C6 crosspeak, and the H8-C2 crosspeak (if present) was overlapped by the strong H8-C4 signal. This finding is consistent with the rather small calculated couplings (Table 1, Entry 3). In the HMBC spectra of compound **4**, the H8-C2 crosspeak was weaker than the H8-C6 crosspeak, which is again in agreement with the calculated coupling constants. We measured the coupled ^{13}C NMR spectrum of compound **5**, and the experimental coupling constants (1.2 Hz for the H8-C6 and 0.4 for the H8-C2 coupling) were in excellent agreement with the calculated values 0.92 and 0.34 Hz, respectively (Table 1, Entry 10). In addition, the H8-C2 crosspeak was missing in the HMBC spectrum.

We obtained the experimental H8-C2 and H8-C6 coupling constants of compound **6** from the coupled ^{13}C spectrum, and again, the calculated values (0.86 and 0.73 Hz, Table 1, Entry 4) agreed

Table 1. The calculated heteronuclear coupling constants *J*(H8-C2) and *J*(H8-C6) in substituted 9- and 7-methylpurines

			9-isomer		7-isomer	
Entry	R^2	R^6	*J*(H8-C2)	*J*(H8-C6)	*J*(H8-C2)	*J*(H8-C6)
1	H	H	0.32	0.36	0.61	0.93
2	Cl	H	0.48	0.34	0.79	0.89
3	H	Cl	0.35	0.21	0.65	0.89
4	Cl	Cl	0.52	0.19	0.86	0.73
5	H	NH_2	0.35	0.55	0.60	0.81
6	NH_2	H	0.57	0.46	0.68	0.96
7	NH_2	NH_2	0.57	0.62	0.68	0.84
8	NH_2	Cl	0.61	0.32	0.75	0.79
9	Cl	NH-CH_3	0.49	0.58	0.76	0.80
10	NH_2	OH[a]	0.34	0.92	0.41	1.2
11	OH[a]	OH[a]	0.36	0.75	0.27	1.1
12	H	OH[a]	0.16	0.85	0.36	1.1
13	Cl	OCH_3	0.49	0.44	0.80	0.73
14	Cl	$N(CH_3)_2$	0.54	0.64	0.79	0.74
15	NH_2	NH-CH_3	0.56	0.66	0.67	0.86

[a]Keto forms of the guanine, hypoxanthine and xanthine bases were used in the calculations.

excellently with the experimental ones (0.8 and 0.7 Hz). Crosspeaks of equal intensity were observed in the HMBC spectrum of this compound. Similarly, in the HMBC spectra of compounds **7**, **9** and **10**, both crosspeaks H8-C2 and H8-C6 were observed, and their intensities were almost identical, which is in line with the calculated coupling constant values 0.7–0.8 Hz for all the couplings. In contrast to that the calculated value of the H8-C2 coupling constant (0.27 Hz) in compound **8** is much smaller than the H8-C6 coupling constant (1.1 Hz), which is, indeed, manifested in the HMBC spectrum by a missing H8-C2 crosspeak.

Based on a careful analysis of the calculated long-range coupling constants, we can conclude that the value of H8-C2 coupling depends primarily on the nature of the substituent in the position 2. The following order of the coupling values depending on the C-2 substituent was observed: $H < Cl < NH_2$. Exceptions from this rule are the keto forms of guanine, hypoxanthine and xanthine, which have always very low H8-C2 coupling. Similarly, the H8-C6 coupling value is dominated by the nature of the C-6 substituent, with the keto forms of guanine, hypoxanthine and xanthine having the highest values of the coupling. 7-methylisomers always have both H8-C2 and H8-C6 coupling constants values higher than the 9-methylisomers.

Conclusions

We have demonstrated both experimentally and by DFT calculations that the four- and five-bond heteronuclear *J*-couplings of the hydrogen H-8 with carbon atoms C-6 and C-2 may be of a comparable size. Depending on the substituents attached to the purine skeleton, the values of the coupling constants can change significantly. For proper structure determination and signal assignment, care must be taken with this issue and one cannot rely on small crosspeaks in the HMBC spectra, which can be caused by both H8-C2 and H8-C6 interactions. The DFT calculated coupling constants were shown to agree very well with the experimental data. An alternative for the structure determination and signal assignment could be also the comparison of calculated and experimental chemical shifts.[20]

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