Pergamon

Tetrahedron 54 (1998) 12243–12248

TETRAHEDRON

Psylloborine A, a New Dimeric Alkaloid from a Ladybird Beetle

Frank C. Schröder[a]* and Till Tolasch[b]

[a]Baker Laboratory, Department of Chemistry,
Cornell University, Ithaca, NY 14853, USA.

[b]Institut für Organische Chemie der Universität,
Martin Luther King Platz 6, D-20146 Hamburg, Germany.

Received 17 June 1998; accepted 31 July 1998

Abstract: A new dimeric alkaloid, psylloborine A (**3**), was identified from the 22-pointed ladybird beetle *Psyllobora vigintiduopunctata* (L.). Psylloborine A exhibits an unusual heptacyclic ring system, derived from two azaphenalene units. Structural assignment was based on NMR-spectroscopic methods, using E.COSY experiments for the analysis of the highly complex spin systems.

Alkaloids play an important role in the defense strategies of arthropods. Especially from ladybird beetles (Coccinellidae) and from ants of the subfamily Myrmicinae, a large variety of defensive alkaloids have been identified [1,2]. In most of these compounds, an usually unbranched carbon chain is attached at one or more sites to a nitrogen atom, forming linear as well as mono-, di-, and tricyclic structures. Recent analyses of the hemolymph from *Chilocorus* and *Exochomus* ladybird beetles led to the identification of a new series of "dimeric" alkaloids (**1**, **2**), the carbon skeletons of which consist of two unbranched chains [3]. Other dimeric and trimeric alkaloids have been identified from the poison gland secretion of *Myrmicaria* ants [4]. Here we report the identification of a new type of dimeric alkaloid, psylloborine A (**3**), from the hemolymph of the 22-pointed ladybird beetle, *Psyllobora vigintiduopunctata* (L.).

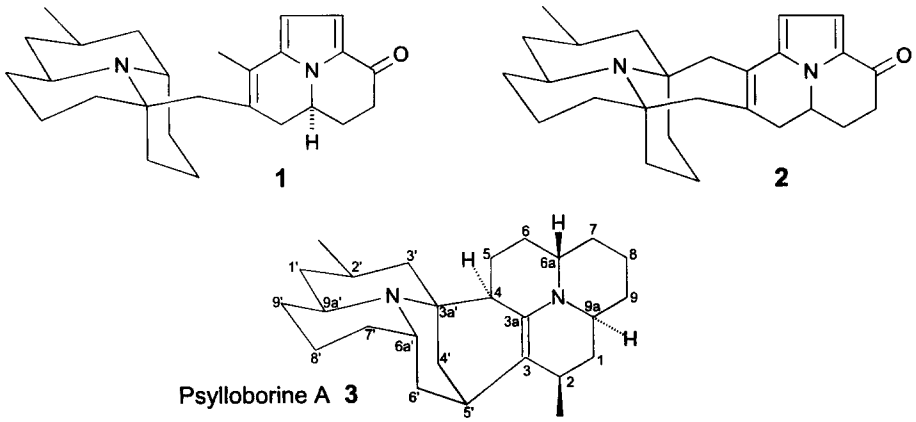

Fig. 1 Dimeric alkaloids from ladybird beetles.

0040-4020/98/$ - see front matter
PII: S0040-4020(98)00753-4

^{1}H and ^{13}C spectroscopic NMR analysis of crude, unfractionated dichloromethane whole-body extracts of *P. vigintiduopunctata* indicated the presence of large amounts of one major secondary metabolite, a mostly aliphatic, oligocyclic alkaloid, in addition to several fatty acids, glycerides and traces of the more lipophilic amino acids. The alkaloid could easily be isolated and was further analyzed by mass spectroscopy and NMR spectroscopy. Positive ion electrospray mass spectroscopy showed singly and doubly protonated pseudomolecular ions at $m/z = 381.3$ and $m/z = 191.1$, suggesting the presence of two basic nitrogen atoms and a corresponding molecular formula of $C_{26}H_{40}N_2$.

Due to the highly congested nature of the proton NMR spectra, the NMR signals of the 40 protons, of which 36 have chemical shift values within a range of less than 2.5 ppm, could not be assigned on the basis of phase-sensitive DQF-COSY spectra alone (Fig. 2). Additional E.COSY experiments [5] were necessary to establish the two large spin systems and to unambiguously determine the proton-proton coupling constants (Table 1). As shown in Figure 3, coupling constants that are difficult to extract from the DQF-COSY spectrum due to overlap or insufficient resolution in F2 can be determined more accurately from the E.COSY spectrum, using crosspeaks at the less crowded periphery of the spectrum. With this approach, most of the vicinal H,H-coupling constants in the two spin systems of psylloborine A (**3**) could be determined.

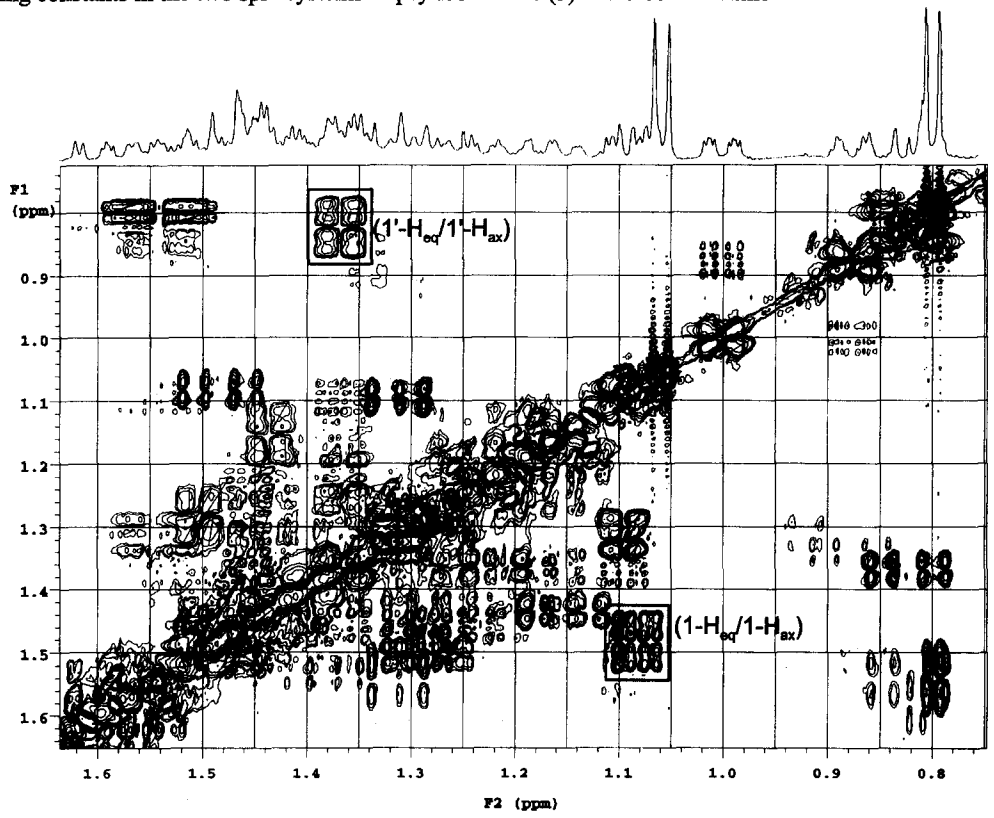

Fig. 2 0.75-1.64 ppm region of the ^{1}H-NMR and the DQF-COSY spectrum of psylloborine A (**3**) (C_6D_6, 500 MHz). The vicinal coupling constants of the proton 1'-H_{eq} (1.37 ppm) can not be directly extracted, due to poor resolution in F2 and overlap with other crosspeaks, e. g. of the proton 8-H_{eq} at 1.36 ppm. The E.COSY signals corresponding to the crosspeaks (1-H_{eq}/1-H_{ax}) and (1'-H_{eq}/1'-H_{ax}) are shown in Fig. 3.

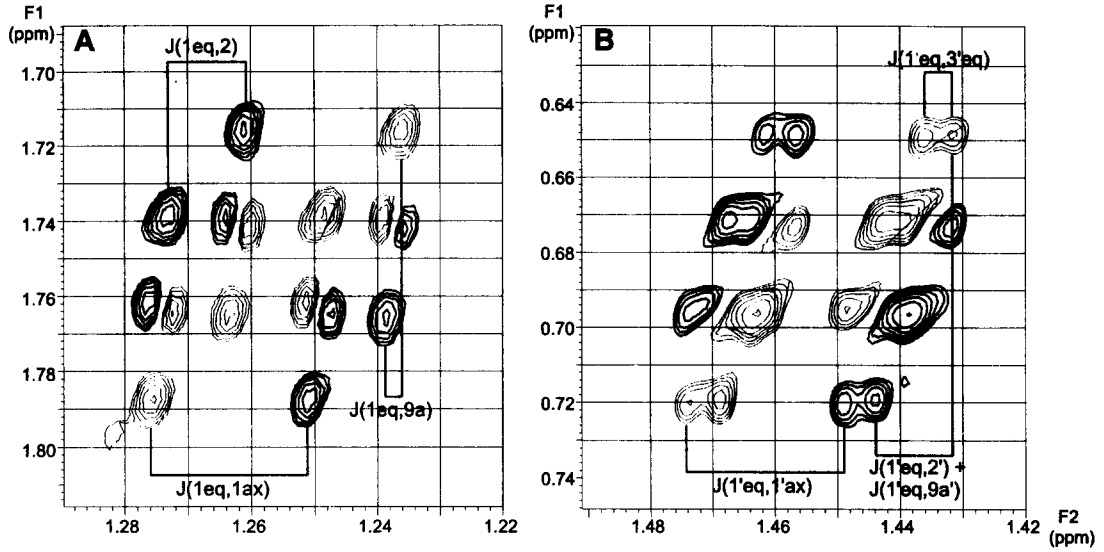

Fig. 3 (**A**): Crosspeak of the geminal pair 1-H_{eq}/1-H_{ax} in the E.COSY spectrum of psylloborine A (**3**) (CD_2Cl_2, 500 MHz). The passive vicinal couplings J(1eq,2) and J(1eq,9a) as well as the active coupling J(1eq,1ax) can be determined without interference from components of opposite phase. (**B**): E.COSY crosspeak of the geminal pair 1'-H_{eq}/1'-H_{ax}. Although, in comparison to the corresponding DQF-COSY spectrum (Fig. 2), the crosspeak is greatly simplified, the passive vicinal coupling constants J(1'eq,9a') and J(1'eq,2') cannot be directly extracted. Due to partial overlap, only the sum J(1'eq,9a')+J(1'eq,2') can be determined. Since J(1'eq,9a') is accessible from the E.COSY crosspeak of 9a'-H/1'-H_{ax} (not shown), J(1'eq,2') can be calculated. The E.COSY crosspeak 1'-H_{eq}/1'-H_{ax} also allows one to determine the active geminal coupling J(1'eq,1'ax). In addition, a small four-bond coupling J(1'eq,3'eq) is revealed.

Because directly detected ^{13}C NMR experiments could not be performed with the amount of material available (220 μg, 0.58 μmol), the chemical shift values of the 26 carbon atoms were determined from sensitivity-enhanced gHSQC [6] and HMBC spectra (Table 1). The identification of the nitrogen-bearing carbon of the enamine moiety (C-3a) proved to be especially difficult. Best results were obtained with a HMBC without gradients using a relatively short delay of 45 ms, which revealed correlations of C-3a to the proton at C-4 (4-H) and to the equatorial proton at C-5 (5-H_{eq}) (Table 1).

The relative configuration of psylloborine A (**3**) was determined using phase-sensitive NOESY spectra and the ($^1H,^1H$) coupling constants (Table 1). The strong 1,3-diaxial NOE's and the coupling constants of the protons of the fully saturated azaphenalene moiety clearly indicate *trans*-fusion of rings **F** and **G** as well as *cis*-fusion of **F/G** and **E** (Fig. 4) Similarly, the *trans*-fused ring system **B/C** was established. The configuration at C-2 and C-9a in ring **A** follow from the NOE-signals 2-H/9a-H and 1-H_{ax}/6a-H. Finally, the configuration at the bridgehead carbon atoms C-4, C-3a', and C-5' can be derived from the strong NOE's 5-H_{ax}/6a'-H and 2-CH_3/6'-H_{eq}. Figure 4 shows the energy-optimized conformation of psylloborine A (**3**) as calculated using the AM1 semi-empirical method.

Table 1 ^{1}H and ^{13}C NMR data of psylloborine A (**3**).

carbon	δ (ppm)[a]	proton	δ (ppm)[b]	J (Hz)[c]	NOE[d]	HMBC[e]
C-1	34.16	1-H_{ax}	1.48	$J_{1ax,1eq}$ = 12.6, $J_{1ax,9a}$=11.8, $J_{1ax,2}$ = 11.4	6a-H	C-2, 2-$\underline{C}H_3$, C-9a
		1-H_{eq}	1.08	$J_{1eq,9a}$ = 1.7, $J_{1eq,2}$ = 5.8	9a-H	C-3
C-2	36.38	2-H	2.32	$J_{2,2CH3}$ = 6.8, $J_{2,4}$ = 1	9a-H	C-1, 2-$\underline{C}H_3$
C-3	119.75					
C-3a	144.94					
C-4	50.57	4-H	2.13	$J_{4,5ax}$ = 12, $J_{4,5eq}$ = 3		C-3a
C-5	24.03	5-H_{ax}	1.64	$J_{5ax,5eq}$ = 12.6, $J_{5ax,6ax}$ = 12.4, $J_{5ax,6eq}$ = 3.9	6a'-H	
		5-H_{eq}	2.06	$J_{5eq,6ax}$ = 3.4, $J_{5eq,6eq}$ = 2		C-3a, C-6a
C-6	35.69	6-H_{ax}	1.56	$J_{6ax,6eq}$ = 12.6, $J_{6ax,6a}$ = 9.7		C-5, C-6a
		6-H_{eq}	1.73	$J_{6eq,6a}$ = 2		C-4
C-6a	56.85	6a-H	2.27	$J_{6a,7ax}$ = 10, $J_{6a,7eq}$ = 3.5		
C-7	34.30	7-H_{ax}	1.16	$J_{7ax.7eq}$ = 12.7, $J_{7ax,8ax}$ = 12.5, $J_{7ax,8eq}$ = 3		
		7-H_{eq}	1.44	$J_{7eq,8ax}$ = 2, $J_{7eq,8eq}$ = 3		
C-8	19.83	8-H_{ax}	1.23	$J_{8ax,8eq}$ = 12.5, $J_{8ax,9ax}$ = 12, $J_{8ax,9eq}$ = 3		
		8-H_{eq}	1.36	$J_{8eq,9ax}$ = 4, $J_{8eq,9eq}$ = 2.5		C-6a
C-9	31.62	9-H_{ax}	1.79	$J_{9ax,9eq}$ = 12.8, $J_{9ax,9a}$ = 6	9a-H	C-1, C-8, C-9a
		9-H_{eq}	1.45	$J_{9eq,9a}$ = 1.3	9a-H	
C-9a	54.32	9a-H	3.21			C-1, C-2, C-6a, C-8, C-9
2-$\underline{C}H_3$	19.96	2-$C\underline{H}_3$	1.06		6'-H_{eq}, 5'-H	C-1, C-2, C-3
C-1'	43.61	1'-H_{ax}	0.82	$J_{1'ax,9a'}$ = 11.5, $J_{1'ax,1'eq}$ = 12.2, $J_{1'ax,2'}$ = 12.2		C-2', 2'-$\underline{C}H_3$, C-3', C-9', C-9a'
		1'-H_{eq}	1.37	$J_{1'eq,9a'}$ = 2.2, $J_{1'eq,2'}$ = 3.9	1'-H	
C-2'	26.86	2-H	1.54	$J_{2',2'-CH3}$ = 6.3, $J_{2',3'ax}$ = 12.2, $J_{2',3'eq}$ = 2		
C-3'	47.20	3'-H_{ax}	1.31	$J_{3'ax,3'eq}$ = 12.8		C-4, C-1', C-2', 2'-$\underline{C}H_3$, C-3a', C-4'
		3'-H_{eq}	1.10			
C-3a'	56.27					
C-4'	30.97	4'-H_{ax}	2.14	$J_{4'ax,4'eq}$ = 12, $J_{4'ax,5'}$ = 3.2	6'-H_{ax}, 9a'-H	C-3, C-4, C-3a', C-5'
		4'-H_{eq}	1.00	$J_{4'eq,5'}$ = 3.2, $^{4}J_{4'eq,6'eq}$ = 1		
C-5'	34.00	5'-H	2.55	$J_{5',6'ax}$ = 3.2, $J_{5',6'eq}$ = 3.2		
C-6'	25.71	6'-H_{ax}	1.93	$J_{6'ax,6'eq}$ = 12.6, $J_{6'ax,6a'}$ = 12.4	9a'-H	C-3, C-5', C-6a'
		6'-H_{eq}	0.88	$J_{6'eq,6a'}$ = 2.4	6a'-H	
C-6a'	48.51	6a'-H	3.49	$J_{6a',7'ax}$ = 6.8, $J_{6a',7'eq}$ = 1.5	7'-H_{ax}, 7'-H_{eq}	
C-7'	31.54	7'-H_{ax}	1.82	$J_{7'ax,7'eq}$ = 12.4, $J_{7'ax,8'ax}$ = 12.2, $J_{7'ax,8'eq}$ = 5		
		7'-H_{eq}	1.39			
C-8	20.55	8'-H_{ax+eq}	1.46	$J_{8'ax,9'ax}$ = 12, $J_{8'eq,9'ax}$ = 5.8		
C-9'	35.55	9'-H_{ax}	1.29	$J_{9'ax,9'eq}$ = 12.4, $J_{9'ax,9a'}$ = 11.2		C-9a'
		9'-H_{eq}	1.50	$J_{9'eq,9a'}$ = 2.4	9a'-H	
C-9a'	49.02	9a'-H	2.67			
2'-CH_3	22.47	2'-$C\underline{H}_3$	0.80			C-1', C-2', C-3'

The subscripts "ax" for axial and "eq" for equatorial refer to the six-membered rings of the two azaphenalene systems. [a]Carbon chemical shift in CD_2Cl_2, determined from gHSQC and HMBC spectra. [b]Proton chemical shift in C_6D_6. [c]Coupling constants determined from DQF-COSY- and E.COSY spectra using CD_2Cl_2 and C_6D_6 as solvents. [d]Positive crosspeaks in phase-sensitive NOESY spectra. [e]Correlations of protons in HMBC spectra acquired in CD_2Cl_2.

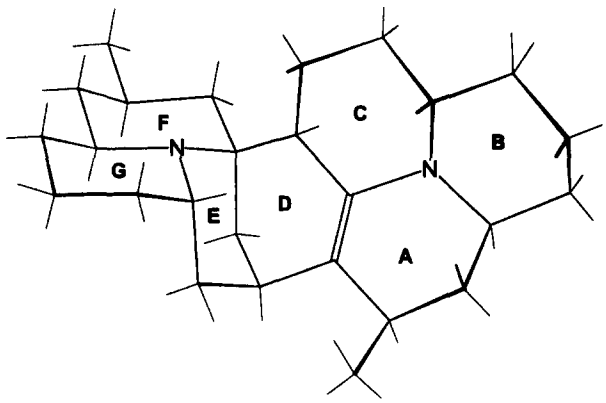

Fig. 4 Conformation of psylloborine A.

Psylloborine A is distinguished from other oligocyclic ladybird alkaloids such as chilocorine and exochomine in that its two unbranched chains of 13 carbon units form two independent 2-methylazaphenalene systems. Thus, psylloborine A can be regarded as a dimer of azaphenalene alkaloids such as precoccinelline (**4**) or propyleine (**5**), which have been identified from the hemolymph of various ladybird species [1]. Interestingly, the hemolymph of *P. vigintiduopunctata* does not appear to contain significant amounts of **4**, **5**, or other azaphenalene monomers.

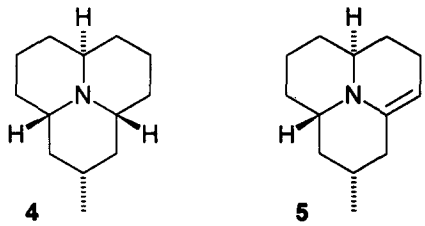

Fig. 5 2-Methylazaphenalenes from ladybird beetles.

EXPERIMENTAL

NMR: Spectra were recorded at 298 K using Varian UNITY+ (500 MHz proton, 126 MHz carbon) and Varian INOVA (600 MHz proton, 151 MHz carbon) spectrometers. CD_2Cl_2 and C_6D_6 were used as solvents. Double quantum filtered COSY (DQF-COSY) and exclusive COSY (E.COSY) spectra were acquired using the standard pulse sequences and phase cycling [5], with usually 512 t_1 values, 64 scans per t_1 increment, and a sweep width of 4 ppm. Phase-sensitive NOESY spectra were acquired with a mixing time of 600 ms, 200 t_1 values, and 64 scans per t_1 increment. HMBC spectra were acquired in the phase-sensitive mode with phase cycling and delays of 45-90 ms (580 t_1 increments, 128-256 scans per t_1 increment). HMBC and E.COSY spectra were acquired using a Shigemi® tube and CD_2Cl_2 as the solvent. - MS: Micromass Quattro (positive ion electrospray ionization). - GC-MS: Gas chromatograph Hewlett Packard HP5890A connected to a Hewlett-Packard MSD (70 eV EI-MS); column: 30 m DB5-MS (J&W Scientific), 0.25 mm i.d., 0.25 μm film.

Isolation of psylloborine A: *P. vigintiduopunctata* adults (8 beetles, both sexes, collected near Hamburg, Germany) were cooled to -20 °C, crushed, and extracted with dichloromethane (3 × 1 ml). After addition of K_2CO_3 (200 mg), the insect bodies were again extracted with CH_2Cl_2 (3 × 1ml). The extracts were combined and concentrated *in vacuo*. The oily residue was then dissolved in 0.6 ml of CD_2Cl_2 and submitted to analysis by NMR spectroscopy, GC/MS, and positive ion electrospray mass spectrometry. Subsequently, the CD_2Cl_2 solution was extracted with 0.1 N aqueous HCl (2 × 1 ml) and the combined aqueous extracts were washed with hexane (3 × 1 ml). After cooling to 0 °C, K_2CO_3 (200 mg) was added and the resulting mixture was extracted with CH_2Cl_2 (3 × 1 ml). The combined organic extracts were dried over K_2CO_3 and then concentrated, yielding 240 μg of a mixture of psylloborine A (**3**) (90%) and a structurally related isomer (10%).

ACKNOWLEDGMENT

We would like to thank Jerrold Meinwald, Ithaca, and Wittko Francke, Hamburg, for helpful suggestions and support. The partial support of this research by the Deutsche Forschungsgemeinschaft grant no. SCHR609/1-1 and by the National Institutes of Health grant no. GM53830 is gratefully acknowledged.

REFERENCES

1. King, A. G.; Meinwald, J. *Chem. Rev.* **1996**, *96*, 1105-1122; Daloze, D.; Braekman, J.-C.; Pasteels, J. M. *Chemoecology* **1995**, *5/6*, 173-183.
2. Braekman, J. C.; Daloze, D. in *Studies in Natural Products*, Vol. 6; Atta-ur-Rahman Ed.; Elsevier: Amsterdam, 1990; pp. 421-466.
3. Timmermanns, M.; Braekman, J.-C.; Daloze, D.; Pasteels, J. M.; Merlin, J.; Declerq, J.-P. *Tetrahedron Lett.* **1992**, *33*, 1281-1284; McCormick, K. D.; Attygalle, A. B.; Xu, S.-C.; Svatoš, A.; Meinwald, J. *Tetrahedron* **1994**, *50*, 2365-2372; Shi, X.; Attygalle, A. B.; Meinwald, J.; Houck, M. A.; Eisner, T. *Tetrahedron* **1995**, *51*, 8711-8718.
4. Schröder, F.; Franke, S.; Francke, W.; Baumann, H.; Kaib, M.; Pasteels, J. M.; Daloze, D. *Tetrahedron* **1996**, *52*, 13539; Schröder, F.; Sinnwell, V.; Baumann, H.; Kaib, M. *Chem. Commun.* **1996**, 2139; Schröder, F.; Sinnwell, V.; Baumann, H.; Kaib, M.; Francke, W. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 77-80.
5. Griesinger, C.; Sørensen, O. W.; Ernst, R. R. *J. Magn. Reson.* **1987**, *75*, 474; Biamonti, C., Rios, C. B., Lyons, B. A., Montelione, G. T. Multidimensional NMR Experiments and Analysis Techniques for Determining Homo- and Heteronuclear Scalar Coupling Constants in Proteins and Nucleic Acids. In *Advances in Biophysical Chemistry*; Bush, C. A. Ed.; JAI Press Inc.: Greenwich, Connecticut, USA, 1994; pp. 51-120.
6. Palmer III, A. G.; Cavanagh, J.; Wright, P. E.; Rance, M. *J. Magn. Reson.* **1991**, *93*, 151-170.