the acidic amino acid(s) in CBP, can also explain the result of (4).[47] Recently, the amino acid sequence of CBP was determined by Rychlik et al.[48] The most unusual feature of the CBP is the high tryptophan content [4.7 mol % (seven residue)], while other initiation factors (eIF-2, -4A, -4B, -4C, 5) vary from 0 to 1 mol % tryptophan.[49] Furthermore, this CBP shows a high content of acidic amino acids (25 and 28 residues for Glu and Asp, respectively). This result adds further weight to the above-mentioned discussions.

Finally, we want to introduce a report[50] that demonstrates the importance of the present results: antibodies specific for methylated DNA elicited in rabbits recognize only a single-strand region of DNA containing m^{7}G base, and its antigenicity is decreased or lost in parallel with the extent of the release of m^{7}G residues. This means that m^{7}G residues of methylated DNA play an important role in forming a characteristic complex with the amino acids of antibody.

The present study clearly shows the importance of the tryptophan residue for selective recognition with the guanine base of nucleic acid. The stacking interactions involving the charge-transfer interactions occur in many biological systems and play a key role in their effective actions.

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Registry No. m^{7}GuA-IAA, 113111-13-0; m^{7}GuO-IAA, 113111-14-1; m^{7}GMP-TRP, 113111-15-2; m^{7}G, 578-76-7; Trp, 73-22-3.

Supplementary Material Available: Tables of final atomic coordinates with isotropic thermal parameters of non-hydrogen atoms, anisotropic thermal parameters of non-hydrogen atoms (m^{7}GuA–IAA and m^{7}GMP–TRP complexes), atomic coordinates and isotropic thermal parameters of hydrogen atoms, bond lengths and angles, least-squares best planes (the atomic deviations from them and the dihedral angles between them), hydrogen bond distances and angles, and ORTEP drawings of molecular conformations and crystal packings (17 pages); tables of observed and calculated structure factors (33 pages). Ordering information is given on any current masthead page.

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[47] This type of hydrogen bond has been found in the m^{7}GMP–L-phenylalanine complex crystals[32] and exists as a predominant form in the solution state: Lancelot, G.; Hélène, C. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4872–4875.

[48] (a) Rychlik, W.; Gardner, P. R.; Vanaman, T. C.; Rhoads, R. E. *J. Biol. Chem.* **1986**, *261*, 71–75. (b) Rychlik, W.; Domier, L. L.; Gardner, P. R.; Hellmann, G. M.; Rhoads, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 945–949.

[49] Grifo, J. A.; Tahara, S. M.; Leis, J. P.; Morgan, M. A.; Shatkin, A. J.; Merrick, W. C. *J. Biol. Chem.* **1982**, *257*, 5246–5252.

[50] Kawarada, Y.; Okuhara, E. *Tohoku J. Exp. Med.* **1986**, *149*, 151–161.

Communications to the Editor

Solid–Liquid Intermolecular Transfer of Dynamic Nuclear Polarization. Enhanced Flowing Fluid ^{1}H NMR Signals via Immobilized Spin Labels

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Although static dynamic nuclear polarization (DNP) experiments for a number of NMR nuclides (e.g., ^{1}H, ^{13}C, ^{31}P, etc.) are well documented for both liquid[1] and solid[2] samples, a number of limitations are inherent in static DNP experiments. This is notwithstanding the potential utility of DNP for providing large Overhauser enhancements (A) which are ultimately proportional to the magnetogyric ratios of the electron and nuclear spins ($A \alpha \gamma_e/\gamma_n$).[1-5]

In most previous DNP studies, inter- and intramolecular effects have been monitored in both static liquid or solid samples in the presence of the free-radical system. However, for many DNP experiments there are undesirable effects caused by the spin label in situ (e.g., line broadening of the NMR signals). In this communication, we demonstrate intermolecular transfer of the polarization from an immobilized spin label to a flowing fluid bolus. *Thus, the flowing liquid bolus (monitored downstream) represents a messenger spatially removed from the immobilized reporter group (i.e., spin label).*

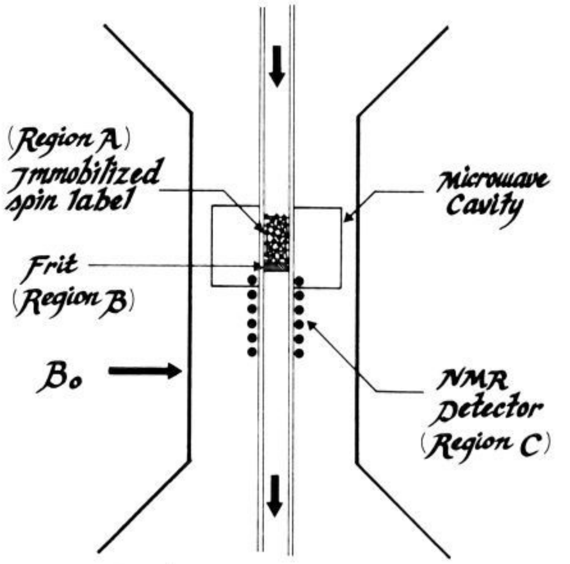

Figure 1. Flow SLIT ^{1}H DNP apparatus. All experiments were performed at $\omega_e/2\pi$ = 9.3 GHz, $\omega_n/2\pi$ = 14.0 MHz, and $B_0 \simeq 3.3$ kg. A modified Varian E-3 EPR spectrometer with a Hughes TWT amplifier was used to generate ω_e in a standard TE$_{102}$ cavity (region A). A wideband JEOL FX-200 spectrometer console was used to detect the ^{1}H NMR signal induced in region C.

We have recently demonstrated[3] a significant advantage of flow DNP in providing independent optimization of the B_{1s} ESR microwave field and the FT NMR detector. For example, for

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[1] For reviews, see: (a) Hausser, K. H.; Stehlik, D. In *Adv. Magn. Reson.* **1968**, *3*, 79. (b) Dwek, R. A.; Richards, R. E.; Taylor, D. *Annu. Rev. NMR Spectrosc.* **1969**, *2*, 293. (c) Müller-Warmuth, W.; Meise-Gresch, K. *Adv. Magn. Reson.* **1983**, *11*, 1.

[2] Wind, R. A.; Duijveshijn, M. J.; Van Der Lugt, C.; Manenshijn, A.; Vriend, J. *Prog. NMR Spectrosc.* **1985**, *17*, 33.

[3] Dorn, H. C.; Wang, J.; Allen, L.; Sweeney, D.; Glass, T. E. *J. Magn. Reson.* **1988**, in press.

[4] Trommel, J. *Ph.D.* Thesis, Delft University of Technology, Delft, Netherlands, 1978.

[5] Natusch, D. F. S.; Richards, R. E.; Taylor, D. *Mol. Phys.* **1966**, *11*, 42.

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a given microwave power, the B_{1s} field at the sample is usually higher in comparison with the conventional static DNP experiment where the NMR coil normally serves as an effective shield of the B_{1s} field (i.e., rf coil at the center of the microwave cavity).[3,4] The flow DNP method also alleviates deleterious sample heating effects by efficient flow removal of a given bolus from the microwave electric field (E_{1s}).

The flow solid–liquid intermolecular transfer (SLIT) DNP experiment is illustrated in Figure 1. The flowing bolus (e.g., benzene) enters region A and develops the nuclear Boltzmann magnetization[5] in a relatively short time (~3–4 T_{1n}) where $(T_{1n})^{-1}$ is given by $(T_{1n})^{-1} = [W_0^D + 2W_1^D + W_2^D + W_0^{sc}]$. W_0^D, W_1^D, and W_2^D are the appropriate transition probabilities for the nuclear–electron dipolar interaction, whereas W_0^{sc} is the transition probability for the nuclear–electron scalar interaction. Also, during this time period, the microwave (B_{1s}) field irradiates the ESR transition(s) of the immobilized radical, and the polarization builds up for the flowing bolus with a time constant T_{1n}.[5] In regions B and C, the polarized bolus relaxes at a relatively slow spin-lattice relaxation rate $(T_{1no})^{-1} = W_{1o}$, where W_{1o} is the transition probability for all other nuclear relaxation modes in the absence of the free radical. Although the volume element for region B in the present experiment is relatively small and the corresponding transfer time (τ_B) is short ($\tau_B < T_{1no}$), in other experiments this may not be the case. The NMR signal for the polarized sample bolus is monitored in region C by using normal FT NMR detection. An important advantage of the flow SLIT DNP experiment is the transfer time τ_B of the sample bolus (to region C) which is now dependent on the less restrictive time (T_{1no}).

In the present experiments, spin labels (**1a**, **1b**, and **2**) (see Scheme I) were immobilized[6,7] on a silica gel surface for the flow SLIT DNP experiments. Immobilized spin labels (**1a** and **1b**) are analogous (except for the type of silica gel support) to the immobilized nitroxide spin label employed by Fyfe and co-workers for lowering spin lattice relaxation times in flowing liquids.[6]

For comparative purposes, flow ^{1}H DNP enhancements (without immobilized radical in region A, Figure 1) were obtained for a 0.01 M solution of 4-hydroxy-TEMPO dissolved in benzene by using ~4 W of microwave power at a flow rate of 8 mL/min. The plot of actual enhancement[8] (A) versus NMR frequency (constant ω_e) is illustrated in Figure 2a. These results are consistent with the work of Bates[9,10] at low field except that the present enhancements are lower by a factor of ~2–3. However, the condition $\omega_e\tau_c < 1$ is undoubtedly not fulfilled in the present higher field experiment.

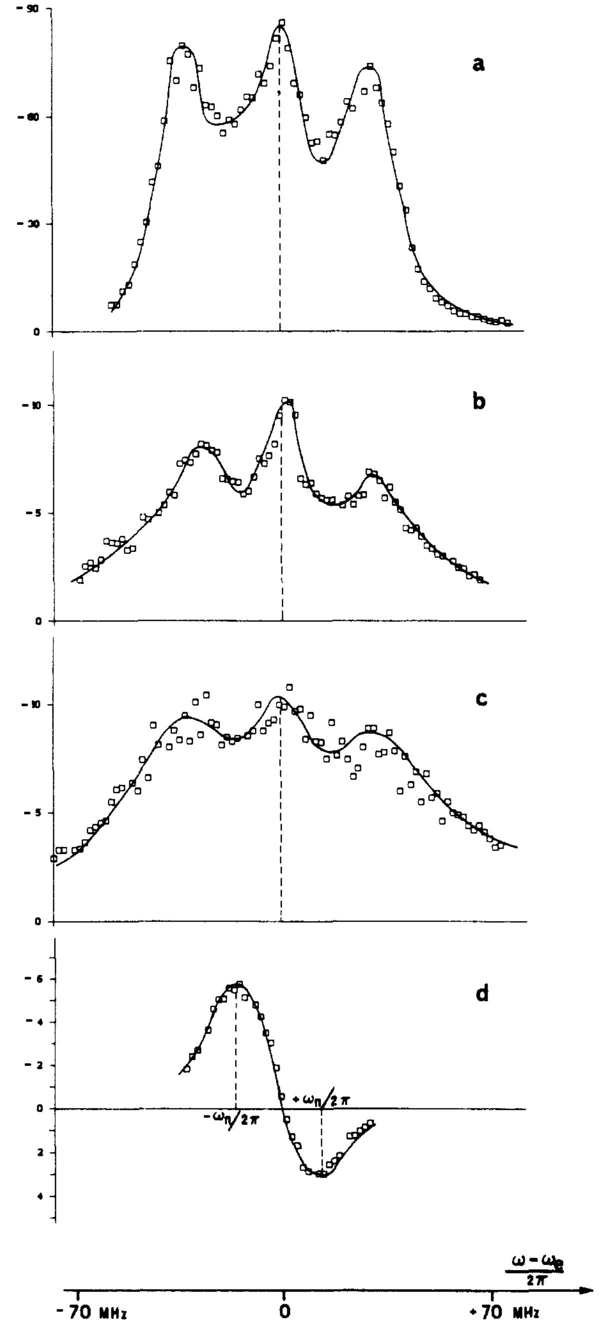

Figure 2. The flow SLIT ^{1}H DNP enhancements (A) versus variable microwave frequency (ω) are plotted for these samples. The actual experiment consists of enhancements (A) versus variable NMR frequency values (constant ω_e, see ref 8). Although different EPR g factors were observed for samples **1a**, **1b**, and **2**, all DNP spectra were normalized to the central line of the nitroxide ^{14}N hyperfine interaction. The enhancements (A) are experimental $[(A_z - A_0)/A_0]$ values at 4 W of microwave power with no correction for the saturation (s) or coupling factor (f). (a) Reference flow ^{1}H DNP enhancements (A) for a flowing solution consisting of 1 × 10^{-2} M 4-hydroxy-TEMPO radical in benzene (no immobilized radical in region A). (b) SLIT ^{1}H DNP enhancements (A) for benzene flowing over immobilized nitroxide spin label **1a**. (c) SLIT ^{1}H DNP enhancements (A) for benzene flowing over immobilized nitroxide spin label **1b**. (d) SLIT ^{1}H DNP enhancements for benzene flowing over immobilized phenoxy spin label **2**. The values at $\omega_e \pm \omega_n$ are indicated.

Shown in Figure 2b–d are the actual flow SLIT ^{1}H DNP enhancements for benzene flowing over the immobilized nitroxide and phenoxy spin labels at a microwave power of ~4 W and a flow rate of 3 mL/min. It should be mentioned that significant

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[6] Immobilized nitroxide radicals **1a** and **1b** were prepared according to procedures published by Bruck et al., (Bruck D.; Duley, B.; Fyfe, C. S.; Delden, J. V.; *J. Magn. Reson.* **1981**, *42*, 51). Immobilized nitroxide sample **1a** was prepared by using silica gel bonded to 30–38 μm glass beads (Whatman). Immobilized nitroxide sample **1b** was prepared with silica gel (Baker, 40 μm). The number of unpaired spins were obtained from EPR measurements according to procedures reported by Swartz et al. Swartz, H. M.; Bolton, J. R.; Borg, D. C. *Biological Applications of Electron Spin Resonance*; Wiley-Interscience: 1972; p 121. The estimated number of unpaired spins was 2.33 × 10^{17} and 3.36 × 10^{17} spins for ~0.1 g of immobilized samples **1a** and **1b**, respectively. In addition, the corresponding EPR line width for the nitroxide central transition was ~4.0 and ~6.0 g, respectively.

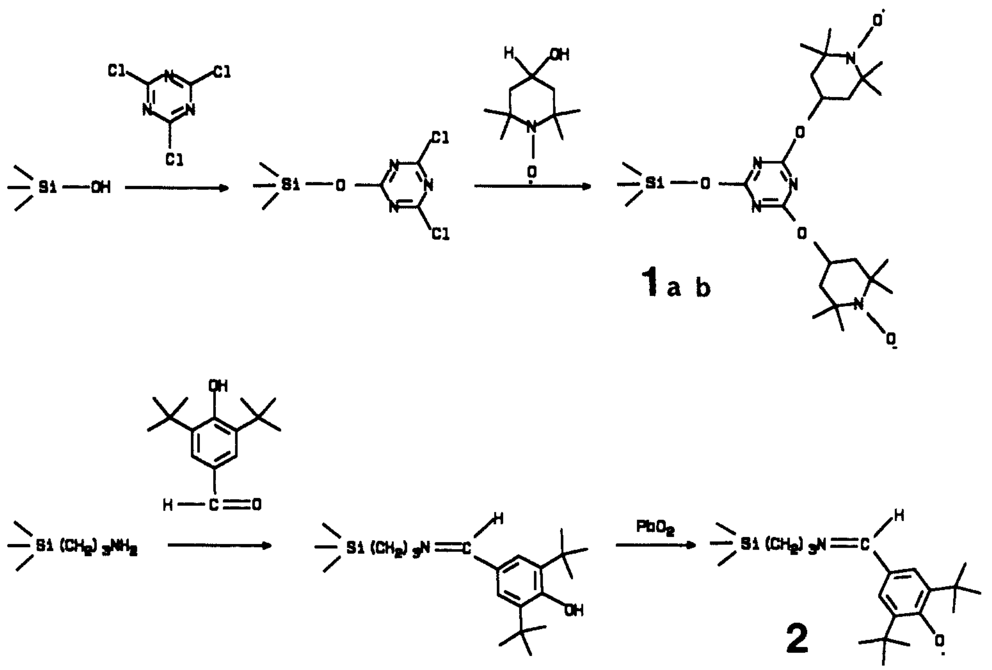
[7] The immobilized sample **2** was prepared by treating 0.5 g of amino capped silica gel (Baker) and 1.0 g of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (prepared according to the procedure given by Cohen (Cohen, L. A. *J. Org. Chem.* **1957**, *22*, 1333)) in a 100-mL, 3-neck flask. About 75 mL of anhydrous benzene was added. The flask was equipped with a magnetic stirring bar and also for soxhlet extraction. The cup was filled with freshly activated 3 Å molecular sieves. The mixture was refluxed for 3 days. Then the derivatized silica gel was filtered and washed several times with hot benzene. The phenoxy radical was activated with PbO$_2$. The number of unpaired spins 0.60 × 10^{17} was measured by EPR (see ref 6) for a 0.1-g sample of the phenoxy immobilized spin label **2**. The corresponding EPR line width was 13.4 g.

[8] Reference to actual enhancements refers to experiments not corrected for saturation (s) or coupling factor (f), but the actual enhancement for the conditions stated. For 4-hydroxy-TEMPO dissolved in benzene, a linear extrapolated A_∞ value of $A_\infty = -80 \pm 5$ was obtained by plotting $[(A_z - A_0)/A_0]^{-1}$ versus (power)$^{-1}$ values for the central nitroxide transition. The $(1/T_2)$DNP line shape was obtained without retuning the resonant LC NMR detector circuit during a given variable field (constant ω_e) experiment. Subtle asymmetry differences for the extrema in Figures 2a–c could be a result of detuning variations of the NMR circuit.

[9] Bates, R. D., Jr.; Drozdoski, W. S. *J. Chem. Phys.* **1977**, *67*, 4038.

[10] Bates, R. D., Jr.; *J. Magn. Reson.* **1982**, *43*, 111.

Scheme I



enhancements occur for flow rates above ~0.5 mL/min. In order to ensure that the measured SLIT DNP enhancements were not due to low level leakage of the spin label from the silica gel surface, ^{1}H nuclear relaxation times were measured. The measured *static* ^{1}H nuclear relaxation times ($T_{1n_0} \simeq 10.5$ s) were not altered after passage through region A containing the immobilized spin labels in comparison with pure benzene (no immobilized radical in region A).[11] Whereas, in the absence of a microwave field ($B_{1s} = 0$) the monitored NMR magnetization did not decrease even at high flow rates (e.g., 10 mL/min), suggesting the high efficacy of these systems in reducing nuclear relaxation times.[7]

The immobilized nitroxide spin label systems **1a** and **1b** have significantly reduced Overhauser enhancements relative to typical liquid values. In addition, the DNP spectral line shape $(1/T_2)_{\mathrm{DNP}}$ is broader for **1b** and **1c** relative to the liquid–liquid intermolecular case (**1a**). These results and the corresponding EPR spectra[6,7] suggest reduced molecular motion for these immobilized spin label/benzene interactions. Furthermore, *the immobilized phenoxy spin label 2 has a dominant solid-state effect* (enhancements at $\omega_e \pm \omega_n$) which is not only completely antisymmetric but also contains a minor Overhauser contribution.[2] This suggests that the immobilized phenoxy spin label **2** has significant contributions from time independent nuclear–electron interactions.

In conclusion, it is important to note the similarity between the present flow SLIT ^{1}H DNP results and data obtained previously for solid ^{1}H DNP studies. For example, the solid-state effect monitored by the messenger group (flowing benzene) for the immobilized phenoxy free radical **2** system clearly distinguishes the present study from previous liquid DNP studies. Since the reporter group reflects properties of the surface, *the flow SLIT DNP experiment could provide a new method for monitoring liquid or gas/surface intermolecular interactions*. An important characteristic of the flow SLIT DNP experiment is the longer nuclear relaxation times (T_{1n_0}) for the flowing bolus after polarization transfer. This more readily facilitates experiments where the flowing bolus is polarized in low magnetic fields (region A), but the NMR magnetization is monitored in higher magnetic fields (region C).[12] The latter experiment is similar in some respects to flow CIDNP experiments[13] where the polarization is generated in low magnetic fields but monitored in higher fields. Similar flow SLIT DNP experiments are presently under study in our laboratory.

Acknowledgment. We gratefully acknowledge helpful discussions with C. S. Yannoni (IBM) and R. A. Wind (CSU) during the early phases of this study. We also gratefully appreciate partial financial support for this work from DOW Chemical Company, Mobil Research Foundation, and the U.S. Army (Ft. Belvoir).

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(11) The nuclear spin lattice relaxation times (T_1's) were measured by using a standard (180°-τ-90°-T) sequence. The T_1's were measured after flowing benzene (degassed and recycled) over the immobilized radical for several minutes and then stopping the flow for the static measurement. The T_1 values were within experimental error of the values obtained for a pure static benzene sample (i.e., 10.5 s).

(12) This experiment was originally suggested by Richards, R. E., et al.: see ref 1b.

(13) For CIDNP reviews, see: (a) Closs, G. L. *Adv. Magn. Reson.* **1974**, *7*, 157. (b) Kaptein, R. *Advances in Free Radical Chemistry*; Williams, G. H., Ed.; Elek Science: London, 1975; Vol. 5, p 319. (c) Wan, J. K. S. *Adv. Photochem.* **1980**, *12*, 283. (d) Lawler, R. G. *Prog. NMR Spectrosc.* **1975**, *9*, 145.

Dependence of the Lifetime of the Twisted Excited Singlet State of Tetraphenylethylene on Solvent Polarity

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Studies on the cis–trans isomerization of olefins have demonstrated the existence of an intermediate (^{1}p*) on the energy surface of the lowest excited singlet state which corresponds to a geometry in which the p-orbitals of the original π-bond are mutually perpendicular.[1] For a symmetrically substituted olefin, this twisted excited singlet state is described by theory in terms of a highly polarizable species whose electronic character is derived from the mixing of zwitterionic structures I and II. On the excited-state surface, ^{1}p* resides approximately vertically above the transition state (^{1}p) on the ground-state surface for the thermal cis–trans isomerization of the alkene. The electronic nature of ^{1}p is described

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(1) See the following reviews and references cited therein: (a) Salem, L.; Rowland, C. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 92. (b) Saltiel, J.; D'Agostino, J.; Megarity, E. D.; Metts, L.; Neuberger, K. R.; Wrighton, M.; Zafiriou, O. C. *Org. Photochem.* **1973**, *3*, 1. (c) Salem, L. *Acc. Chem. Res.* **1979**, *12*, 87. (d) Saltiel, J.; Charlton, J. L. *Rearrangements in Ground and Excited States*; de Mayo, P., Ed.; Academic Press: New York, 1980; Vol. 3, p 25. (e) Hochstrasser, R. M. *Pure Appl. Chem.* **1980**, *52*, 2683. (f) Rettig, W. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 971. (g) Bonačić-Koutecký, V.; Koutecký, J.; Michl, J. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 170.

0002-7863/88/1510-2296$01.50/0