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Host–Guest Complexes as Water-Soluble High-Performance DNP Polarizing Agents

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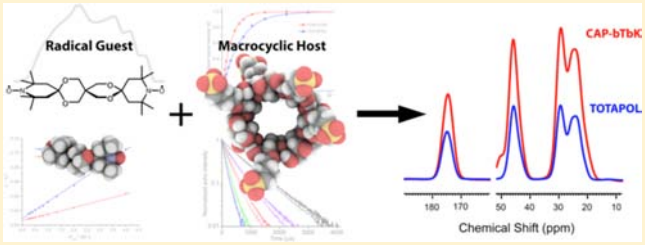
Supporting Information

ABSTRACT: Dynamic nuclear polarization (DNP) enhances the sensitivity of solid-state NMR (SSNMR) spectroscopy by orders of magnitude and, therefore, opens possibilities for novel applications from biology to materials science. This multitude of opportunities implicates a need for high-performance polarizing agents, which integrate specific physical and chemical features tailored for various applications. Here, we demonstrate that for the biradical bTbK in complex with captisol (CAP), a β-cyclodextrin derivative, host–guest assembling offers a new and easily accessible approach for the development of new polarizing agents. In contrast to bTbK, the CAP-bTbK complex is water-soluble and shows significantly improved DNP performance compared to the commonly used DNP agent TOTAPOL. Furthermore, NMR and EPR data reveal improved electron and nuclear spin relaxation properties for bTbK within the host molecule. The numerous possibilities to functionalize host molecules will permit designing novel radical complexes targeting diverse applications.



INTRODUCTION

Dynamic nuclear polarization (DNP) has been demonstrated to boost significantly nuclear polarization efficiency and, therefore, sensitivity in solid-state NMR (SSNMR) even at high magnetic fields by orders of magnitude.[1,2] As a result, new SSNMR applications on highly challenging and intriguing chemical and biological systems become feasible, such as structural investigations on functionalized material surfaces,[3,4] active catalytic sites,[5] polymers,[6] membrane proteins and amyloid fibrils,[7−11] and even biomolecular machineries.[12−14] Several distinct mechanisms could contribute to DNP processes in solids.[1] Among them, the cross-effect (CE) has yielded the highest DNP polarization enhancement at high fields. CE is a three-spin process, which transfers polarization from microwave-pumped, dipole-coupled electron pairs to an adjacent nucleus. The electron–electron dipolar interaction has to be sufficiently strong, and the differences in the precession frequencies of two electron spins has to match the nuclear Larmor frequency.[15,16] On the basis of these conditions, biradicals have been introduced, in which the interspin distances between two unpaired electrons are restricted.[17] One of the most widely used CE DNP biradicals is TOTAPOL (Scheme 1a).[18] It was found that rigid nitroxyl biradicals with almost perpendicularly oriented *g*-tensor principal axes of both unpaired electrons (e.g. *bis*-TEMPO-*bis*-Ketal (bTbK) and its derivatives (Figure 1b,c))[19−21] further improve the frequency matching in randomly oriented samples such as frozen glassy solutions[19] or dried powders.[22,23] Furthermore, substitution of methyl groups on TEMPO moieties, as well as increased molecular weight, enable an even better electron spin saturation by slowing their electron relaxation processes as shown for bCTbK and TEKPol (Figure 1c). These agents work well for currently routinely achievable microwave powers and sample temperatures.[22,24] Besides DNP performance, water solubility is also one of the main boundary conditions for the development of new radicals for biological DNP SSNMR applications.[18,25] The ideal CE DNP polarizing agents should integrate all of the above features. Recently, exciting progress has been made toward this goal, which requires chemical modifications, or even extensively redesigning the molecular frameworks for obtaining proper solubility, electron–electron distances, *g*-tensor orientations, and relaxation properties.[16]

Here, we use bTbK as an example to demonstrate a new strategy for building up new high-performance DNP polarizing agents by assembling them into host–guest complexes, which show improved water-solubility and altered relaxation properties while preserving the radical core structure.

RESULTS AND DISCUSSION

The rigid biradical bTbK in DMSO/D_2O/H_2O (77:17:6) solvent yields significantly higher DNP enhancement than TOTAPOL, a widely used water-soluble polarization agent for biomolecular DNP SSNMR. It, therefore, provides an attractive

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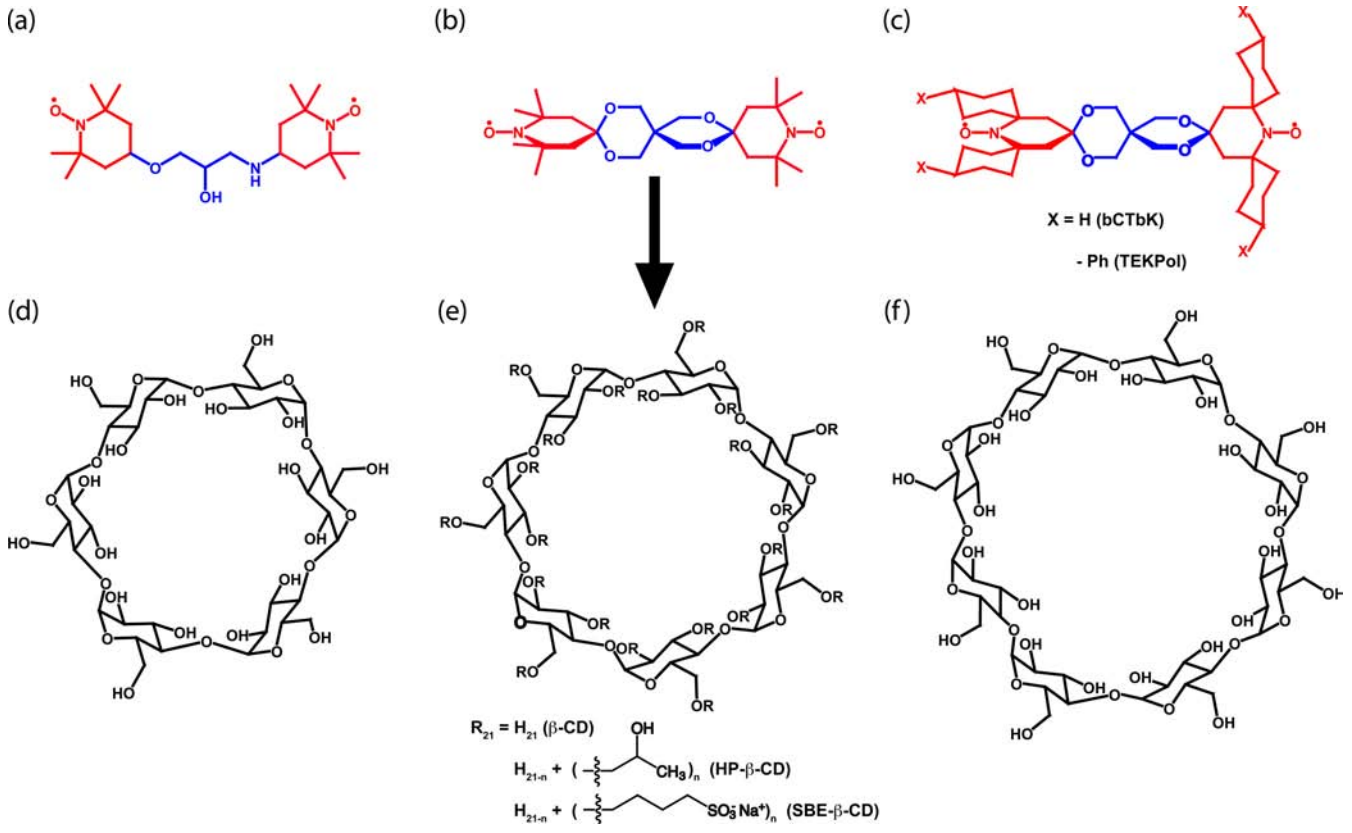
Scheme 1. Structures of various radicals (a–c) and host molecules (d–f). (a) TOTAPOL,[18] (b) bTbK,[19] (c) bCTbK and TEKPol,[22,24] (d) α-cyclodextrin, (e) β-cyclodextrin and its derivatives (HP-β-CD and SBE-β-CD), and (f) γ-cyclodextrin. The red parts in (a–c) show the TEMPO and *bis*-spiro-cyclodexyl TEMPO. The blue parts in a–c present the linkers for building up biradicals.



template for DNP agent development. Unfortunately, bTbK is highly insoluble in water or water-containing glasses due to its bulky and hydrophobic trispiro bisketal linker (shown in blue in Scheme 1b), which prevents most applications to biological systems. Recently, several groups have reported on attempts on improving its solubility through chemical modifications.[20,21] An alternative and general strategy for dissolving hydrophobic organic compounds is assembling them into supramolecular complexes with soluble host molecules. In particular, there has been a growing interest in the formation of inclusion complexes of radicals with various hosts.[26−31] We, therefore, took advantage of host–guest chemistry and tested the solubility of bTbK in aqueous solutions of a variety of potential host molecules. Cyclodextrins (CD, Scheme 1d–f), a family of macrocyclic molecules constituted by glucopyranoside units, have been chosen because they are often highly water-soluble, chemically inert, biologically compatible, and are already widely used on many occasions including pharmaceutical formulation as well as membrane protein reconstitution.[32]

Upon vortex-mixing, bTbK is gradually dissolved at concentrations up to about 20 mM in aqueous solutions of β-CD modified with hydroxypropyl groups (HP-β-CD, Scheme 1e) or sulfobutyl groups (SBE-β-CD, commercial name ***Captisol***, Scheme 1e) under neutral pH (Table 1). The observed solubility, which clearly indicates the formation of soluble complex(es) of bTbK with β-CD molecules, is remarkably higher than that in pure water or in a water–glycerol matrix and is even higher than its recently developed soluble versions.[21] Under alkaline conditions, unmodified β-CD is already able to dissolve bTbK (Table 1). This indicates that the improved solubility is mainly a result of the accommodating of bTbK into the internal cavity of β-CD. On the other hand, as reflected by the host/guest ratio (Table 1), the presence of substitution groups on host molecules further improves the solubility of bTbK, suggesting extra contributions from these groups in organizing a proper binding environment for the bTbK molecule. Further tests have shown that bTbK remains insoluble in α- or γ-CD, which has a smaller or larger interior compared to β-CD. The width of the bTbK molecule defined by the ^{1}H–^{1}H distance between the methyl groups on the TEMPO moiety is about 6.4 Å. This value is very close to the diameter of the large opening (2,3-OH rim) of the β-CD ring but is about 1 Å larger than the large opening of α-CD and about 1 Å smaller than the small opening (6-OH rim) of γ-CD (Table 1). Therefore, the size matching, which permits a proper fitting of the bTbK molecule into the host cavity, is one of the key reasons for the optimal interaction of bTbK with β-CD molecules.

After establishing solubility, we examined the DNP ^{1}H polarization enhancement (ε) using these new bTbK–cyclodextrine agents at 103 K and 9.2 T (392.8 MHz of ^{1}H Larmor frequency, 258.7 GHz of free electron Larmor frequency) through ^{1}H–^{13}C cross-polarization (CP) experiments. All DNP SSNMR tests were performed at 8 kHz MAS frequency, which was frequently used in our group for a variety of SSNMR experiments on membrane proteins.[10−12] HP-β-CD was ruled out after initial measurements because of the severe NMR signal quenching possibly caused by high concentration of

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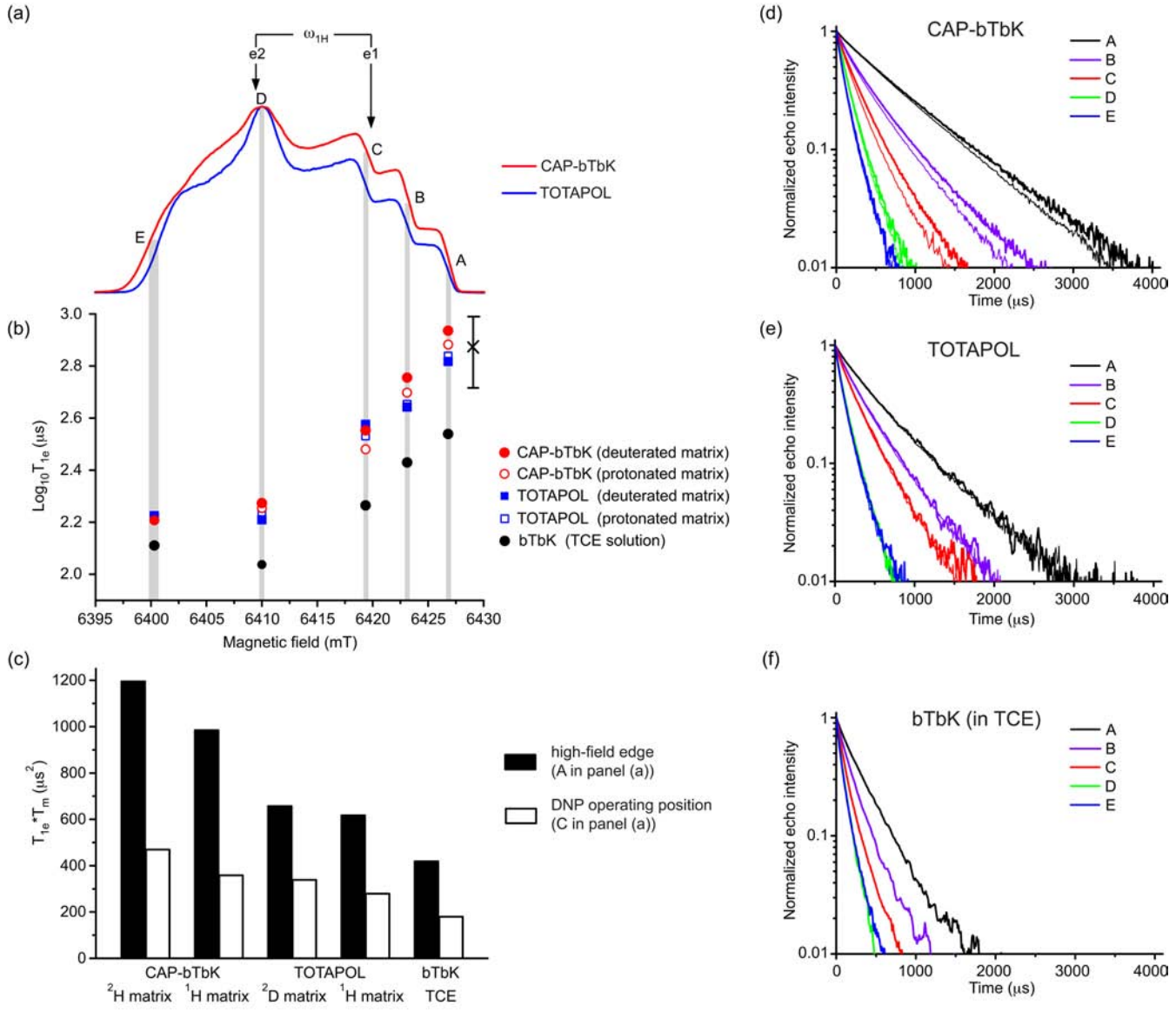

Figure 1. G-band EPR (180 GHz) characterizations of DNP agents. (a) Pulsed EPR spectra of CAP-bTbK and TOTAPOL (9 mM biradical, 18 mM unpaired electron) in a deuterated matrix. The field positions corresponding to *g*-tensor principal axis values g_{zz} ($m_I(^{14}N) = -1, 0, +1$), g_{yy}, and g_{xx} are labeled A–E, respectively (see Supporting Information for details). The approximated field positions relevant in ^{1}H DNP NMR experiments are indicated with e1 and e2, where e1 represents the field of maximum SSNMR signal enhancement and e2 marks the field position of the second electron fulfilling the CE matching condition. The largest positive DNP enhancement is achieved if the separation between e1 and e2 corresponds to the ^{1}H nuclear Larmor frequency. (b) Longitudinal electron spin relaxation times (T_{1e}) of CAP-bTbK, TOTAPOL, and bTbK (in TCE) in frozen radical solutions (9 mM biradical, 18 mM unpaired electron) at 100 K and various field positions. The plots for different agents are aligned at the same field positions in order to provide a more visible comparison. The bar in the right of the panel indicates the estimated errors in measured T_{1e} values (± 30%). (c) $T_{1e}{\cdot}T_m$ of DNP agents at the high-field edge and the e1 position (A and approximately C position in panel a). (d–f) Time traces from electron saturation–recovery T_{1e} measurements. The echo intensity was defined as $[I(\infty) - I(t)]/[I(\infty) - I(t_0)]$, where $I(t_0)$, $I(t)$, and $I(\infty)$ are the EPR signal intensity at the initial data point, at time *t*, and at long recovery time ($>5{\cdot}T_{1e}$), respectively.

Table 1. Solubility of bTbK in aqueous solutions of various cyclodextrins

host molecule							
CD ring	chemical modification	degree of substitution[a]	large rim diameter (nm)	small rim diameter (nm)	host concentration (mM)	bTbK solubility (mM)	host/bTbK molecular ratio
α-CD	(none)		0.53	0.47	133	<1	>130
β-CD	(none)		0.65	0.60	220	~ 10[b]	22
	hydroxpropylation	65%	0.65	0.60	259	20	13
	sulfobutylation	31%	0.65	0.60	230	18	13
γ-CD	(none)		0.83	0.75	192	<1	>190

[a]Averaging values. [b]In saturated ammonia solution; approximate value due to instability of the solution.

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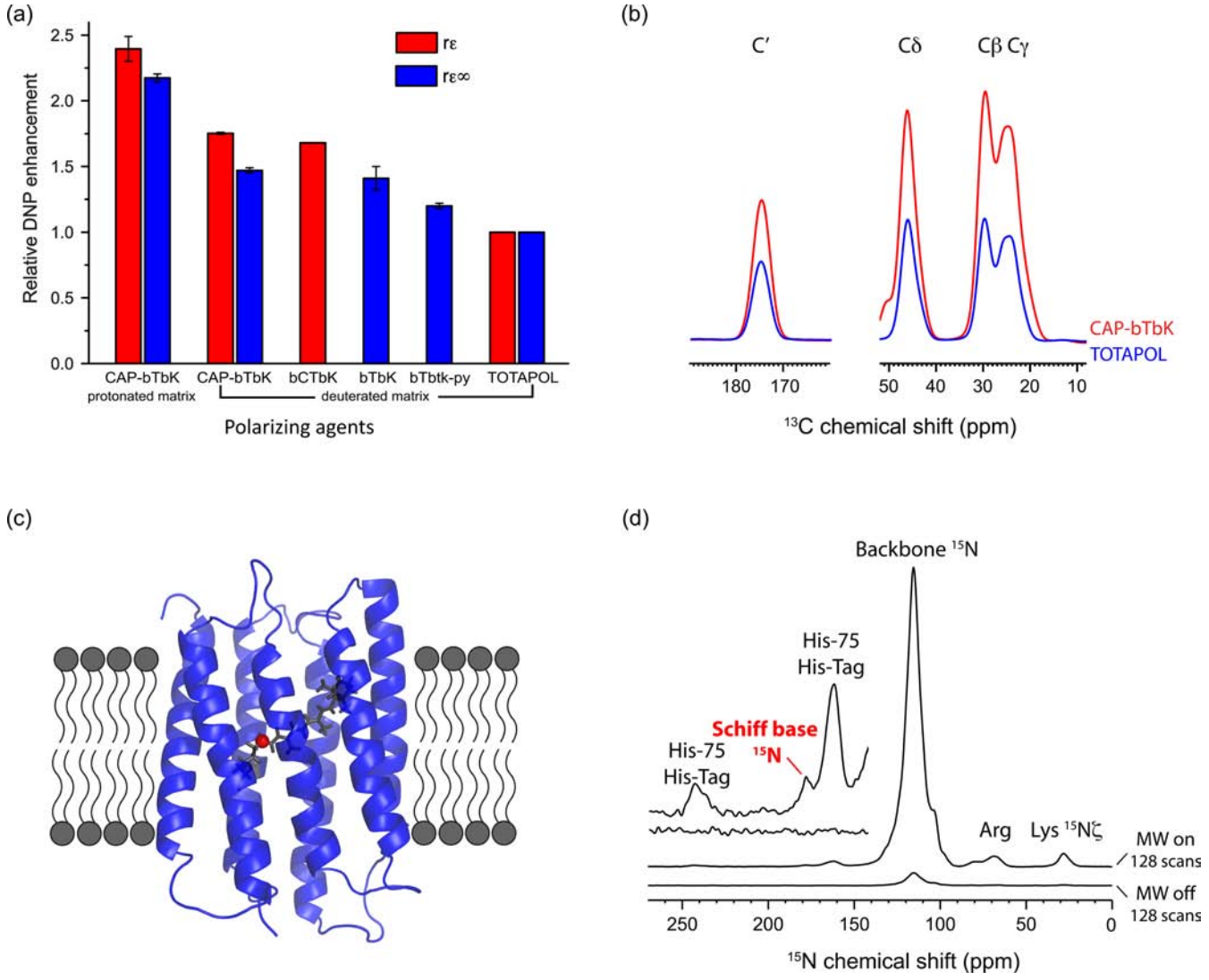

Figure 2. (a) Comparison in relative DNP enhancements by different polarizing agents in reference to TOTAPOL. The values obtained under selected microwave power (r_ε) are shown in red, and the ratios of extrapolated enhancements at infinite power ($r_{\varepsilon,\infty}$) are shown in blue. All the ^{1}H polarization enhancements were measured indirectly through CP. The bCTbK data from literature[22] was recorded on a different sample. The $r_{\varepsilon,\infty}$ for bTbK and bTbtk-py is reported in references.[19,21] (b) Comparison in effective signal-to-noise gain of DNP ^{1}H−^{13}C CP SSNMR experiments using CAP-bTbK or TOTAPOL as polarizing agents, respectively. ^{13}C-labeled proline was used in these tests. The interscan delays were optimized to 1.3 times of T_{1H} and two spectra were acquired with same experimental time. The spectra were calibrated according to number of scans in order to keep the noise in two spectra at the same level. CAP-bTbK sample shows about 80% increase in obtained ^{13}C′ signal intensity. (c) A structural model of proteorhodopsin with Schiff base nitrogen highlighted in red. (d) DNP-enhanced ^{1}H−^{15}N CP spectra of ^{15}N uniformly labeled proteorhodopsin (27 kDa, 5 mg) in a lipid environment using 10 mM CAP-bTbK as polarizing agent (enhancement ε = 24). The inset shows the Schiff base region.

methyl groups introduced by the host.[33] Finally bTbK dissolved in SBE-β-CD solution (CAP-bTbK), an FDA-approved pharmaceutical solubilizer, was selected for further tests. The high-field G-band (6.4 T, 180 GHz) EPR spectra show that the g-tensor principal values as well as the hyperfine interaction in CAP-bTbK are very similar to those in TOTAPOL (Figure 1a, Supporting Information Table S1). Therefore, the microwave frequency optimized for TOTAPOL was also used for all DNP measurements on this new agent. A signal enhancement ε of 60 for TOTAPOL, but 110 for CAP-bTbK, at a concentration of 9 mM under a microwave power of 4 W was observed (Supporting Information Table S2). For easier comparison of the DNP performance of CAP-bTbK or other new agents, we define the relative DNP enhancement $r_\varepsilon = \varepsilon_{agent}/\varepsilon_{TOTAPOL}$. The enhancement factors are, to a certain extent, also affected by the nature of the solvent matrix. Therefore, r_ε compares in a practical way the DNP performance of different radicals in their corresponding solvents. Here, CAP-bTbK yields r_ε of 2.4 and 1.8 in a protonated and deuterated glycerol/water matrix, respectively (Figure 2a, Supporting Information Table S2). Previous work on bCTbK dissolved in tetrachloroethane (TCE),[22] in which the bTbK structure was directly modified, resulted in a r_ε comparable to CAP-bTbK in aqueous matrix (Figure 2a). Recent DNP data on AMUPOL,[25] developed by two of the authors in this work and their co-workers, show superior r_ε values of 3.36 in glycerol/water matrix. Noticeably, complicated radical structural modifications as well as intensive synthetic work are required for constructing such radicals.

Besides the factors discussed above, DNP performance depends on the efficiency of electron spin saturation, which is determined by technical imperfections (limited microwave power, nonresonating cavity, relatively high sample temper-

atures) and relaxation properties of the electron−nuclei spin system. In order to compare the performance of our polarizing agents with those reported in the literature and recorded under a multitude of different conditions, we have determined the DNP enhancements ε at different microwave powers (P). The resulting linear P^{-1} dependence of $(\varepsilon - 1)^{-1}$ (see Supporting Information Figure S3) agrees well with previous observations.[1,21,34] The extrapolated enhancement at infinite microwave power (ε_∞) extracted from this plot partially eliminates variations in electron spin saturation levels due to differences in instrumentation and radical/sample properties. Subsequently, the relative DNP enhancement at high microwave power is defined as above ($r_{\varepsilon,\infty} = \varepsilon_{\infty,\text{agent}}/\varepsilon_{\infty,\text{TOTAPOL}}$). We have obtained an ε_∞ of 107 and 166 for 9 mM TOTAPOL and CAP-bTbK samples in deuterated glycerol/water matrices, respectively (Figure 2a, Supporting Information Table S2). The relative enhancement $r_{\varepsilon,\infty}$ for CAP-bTbK in glycerol/water mixture is found at 1.47 and is even slightly higher than that reported on free bTbK (1.41) (Figure 2a, Supporting Information Table S2).[19]

For an optimal electron spin saturation under microwave irradiation, long longitudinal (T_{1e}) and transverse (approximated by phase memory time T_m) electron spin relaxation times are required.[35] We have measured both parameters for the DNP agents used here at various positions across the EPR profile at high field (6.4 T). As shown in Figure 1b,d−f and Supporting Information Table S3, CAP-bTbK features a larger T_{1e} at all probed field positions compared to free bTbK. This is possibly due the increased size of radical while coupled to a large host, the hindrance of radical methyl group rotation upon host−guest interactions, or the depletion of rotating groups (CD_3, $CHCl_2$) in the organic solvents.[36] It was also observed that T_m of bTbK increases significantly within the host−guest complex (Supporting Information Figure S2 and Table S3). The product $T_{1e}\cdot T_m$ defines the electron saturation factor. For CAP-bTbK, it is about twice as high compared to TOTAPOL and 2.7 times higher compared to bTbK at the high field edge of the EPR spectrum (position A in Figure 1a, Figure 1c). At the DNP operating field position (DNP microwave pumping position, "e1" in Figure 1a), $T_{1e}\cdot T_m$ in CAP-bTbK is at least two times larger than free bTbK and still close to TOTAPOL (Figure 1c). These values suggest a better saturation of electron spins in CAP-bTbK under DNP conditions. Indeed, we have found for CAP-bTbK that r_ε is larger than $r_{\varepsilon,\infty}$ (Figure 2a, Supporting Information Table S2). This means that electron spins in CAP-bTbK can be better saturated at lower microwave power compared to TOTAPOL. However, the r_ε of free bTbK slightly drops in comparison with its $r_{\varepsilon,\infty}$.[19] The slowed electron spin relaxation processes of a radical inside a host molecule generate extra benefits for DNP enhancement besides the unperturbed radical structures.

At the low temperatures commonly used for CE DNP SSNMR, the extended nuclei relaxation times (T_{1H}) and polarization build-up times (T_B) become a practical issue limiting the repetition rate of data acquisitions and therefore decrease the effective spectral sensitivity per unit time. Fortunately, the polarizing agents used in DNP SSNMR experiments also act as paramagnetic dopants for breaking these limits. We found that T_{1H} of a 9 mM CAP-bTbK sample in a deuterated matrix is remarkably shorter than for TOTAPOL under the same conditions (4.1 vs 8.4 s as shown in Supporting Information Table S2). Comparing CAP-bTbK in a protonated with a deuterated matrix reveals a clear decrease in T_{1H} (7.8 vs 4.1 s), whereas TOTAPOL remains almost unchanged (8.1 vs 8.4 s, Supporting Information Table S2). Under the experimental conditions used here, T_{1H} is mainly determined by paramagnetic effects due to the presence of radicals.[37] The radical relaxation properties are barely affected by matrix deuteration/protonation as shown for T_{1e} measured across the EPR profile for both CAP-bTbK and TOTAPOL (Figure 1b and Supporting Information Table S3). Therefore, the observed differences in T_{1H} induced by matrix deuteration are caused by other effects. The host molecule carrying bTbK remains largely protonated in a deuterated matrix due to the presence of many more nonexchangeable sites on its glucose unit (102 per host molecule in average) compared to exchangeable hydroxyl protons (14 per host molecule in average). This results in high local proton density surrounding the radical entrapped in the host molecule even in highly deuterated solvents. These protons experience severe paramagnetic relaxation enhancement (PRE) or electron dipole−dipole reservoir (EDDR)-driven nuclear relaxation. In the solid state, the measured T_{1H} is an average over the ^{1}H pool, since the ^{1}H−^{1}H spin diffusion (SD) occurs much faster than the longitudinal ^{1}H spin relaxation and is supported by a single-exponential relaxation behavior crossing the whole NMR spectra. In the case of CAP-bTbK in a deuterated matrix, fast relaxing protons surrounding the radical contribute more to the overall T_{1H} than in protonated samples, explaining the observed differences.

Another aspect to consider is the effect of radicals on SD: protons located very closely to the paramagnetic centers could experience marked hyperfine interactions and, therefore, SD processes involving these nuclei might be inhibited.[38] However, it has been shown that the dimension of the SD barrier could be less than 1 nm,[37,39] which is still significantly smaller than the outer diameter of β-CD (1.66 nm). In addition, the sulfobutyl groups in SBE-β-CD molecules may further expand the size of host molecules out of the SD barrier. Moreover, it has been pointed out that MAS drives the crossing-over of resonating frequencies and, therefore, compensate the SD quenching.[40,41] These findings provide further supports for the assumption that fast-relaxing ^{1}H nuclei in CAP-bTbK samples could participate in SD and tune down the average T_{1H} in the sample. We expect that a similar tuning effect on T_{1H} could also be achieved by covalently attaching large groups onto a radical, which could be an interesting aspect for developing DNP agents and in general paramagnetic dopants for fast NMR data acquisition.

Knowing T_{1H} enabled us to use optimized interscan delays in our DNP SSNMR experiments, which were set to 1.3 times T_{1H}. As shown in Figure 2b, the optimized sensitivity per unit time is about 80% to 100% higher than that by TOTAPOL and is also significantly better than for free bTbK. This gain is consistent with the results calculated from the values in Supporting Information Table S2, also taking quenching factors into account. On a large seven-transmembrane proton pump proteorhodospin reconstituted in lipid environment,[11,42−45] 10 mM CAP-bTbK results in a 24-fold DNP signal enhancement (Figure 2c), which outperforms the enhancement obtained by 20 mM TOTAPOL under identical sample preparation conditions.[11] This good DNP enhancement permits the observation of different types of nitrogen sites (Figure 2c), including the protonated Schiff base, within 10 min using only a few milligrams of sample. These results indicate that our strategy is rather promising not only for endowing radicals with

new physical or chemical characteristics but also for directly improving their DNP performances on different samples.

It is worth noting that a significant orientation dependence of T_{1e} times has been found in both 9 mM TOTAPOL and CAP-bTbK samples at G-band frequency (Figure 1b). While CAP-bTbK is diluted to 0.5 mM (1 mM electron concentration), T_{1e} still varies in a similar manner (Supporting Information Table S3). Moreover, this phenomenon even remains in 1 mM 4-hydroxy-TEMPO (TEMPOL) monoradical samples at high field. These data preliminarily rule out the contributions from the intramolecular and intermolecular electron−electron dipolar coupling in the observed orientation dependence in T_{1e}. A similar anisotropic character of electron T_1 relaxation has also been observed on TEMPO samples at W-band frequency (94 GHz, 3.4 T) and has been attributed to the anisotropic vibrational modulation of the spin−orbital coupling.[46,47] The detailed mechanisms governing the electron relaxation under DNP conditions might be rather complex and remain an interesting subject for further investigations. Nevertheless, the data presented here reveal a necessity in including this variation into spin dynamics simulations for DNP at high-fields, since the T_{1e} is significantly different at varying resonance positions as shown in Figure 1. In addition, the marked orientation dependence of T_{1e} may partially explain the asymmetry in field dependent DNP enhancement profile of radicals.[19]

CONCLUSION

In conclusion, host−guest assembling offers a feasible and complementary platform for creating new high-performance DNP SSNMR polarizing agents by gaining improved water solubility, proper molecular and electronic structures, and favored electron and nuclei relaxation behaviors at the same time. Our results should promote the further explorations and developments of the rich supramolecular chemistry targeting the engineering of self-assembled DNP polarizing agents for different applications in future. The numerous possibilities to functionalize host molecules will permit designing novel radical complexes with tailored chemical and biochemical features as needed for diverse applications.

MATERIALS AND METHODS

DNP MAS SSNMR experiments were performed on our home-built system based on a Bruker Avance II wide-bore spectrometer operated at 392.80 MHz and equipped with a modified triple resonance 3.2 mm cryo-MAS probe head. Microwaves were generated from a gyrotron (Gycom, Russian Federation) operated at second harmonic mode and were directed into the probe head through broadband corrugated waveguides constructed by the Academy of Science (Kiev, Ukraine).[48] EPR studies were performed on a home-built high-frequency pulsed EPR spectrometer.[49,50] The operating frequency of the spectrometer is 180 GHz (G-band), and the static magnetic field is approximately 6.422 T. Further details are given in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Materials and methods including sample preparation details, DNP SSNMR, G-band EPR, electron spin relaxation data analysis. Table S1: g-tensor principal values for TOTAPOL and CAP-bTbK. Table S2: summary of DNP SSNMR results of CAP-bTbK and TOTAPOL. Table S3: T_{1e} and T_m of various agents. Table S4: electron relaxation time analysis; Figure S2: electron T_m traces; Figure S3: $(\varepsilon - 1)^{-1}$ vs P^{-1}. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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