JOURNAL OF MAGNETIC RESONANCE, Series B **108**, 274–275 (1995)

COMMUNICATIONS

N,N',N''-Trimethyl(2-trimethylsilyl)ethylammonium Iodide: A Universal Internal Standard for ^{1}H, ^{13}C, and ^{15}N NMR*

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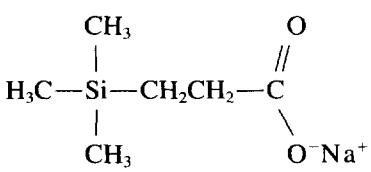
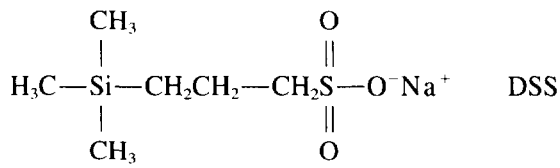
Received April 24, 1995; revised May 31, 1995

Nuclear magnetic resonance spectroscopy is now playing an important role in the determination of the three-dimensional structures of proteins in solution (*1, 2*), and with the recent development of novel theoretical approaches (*3–6*), the isotropic chemical shift is now being used in structure prediction and refinement (*3, 4, 7, 8*). Unfortunately, considerable uncertainty exists in most reported ^{1}H, ^{13}C, and ^{15}N chemical shifts, due to differences in referencing methods used by different research groups. These effects appear to originate from the haphazard use of internal and external references, differences in sample geometry, the use of coaxial references, and the pH and temperature dependences of many common standards. Errors in ^{13}C chemical shifts of up to ~2 ppm, and errors in ^{15}N shifts of up to 4 ppm, are common in protein NMR work (*9*). It would therefore be desirable to be able to use a universal internal standard for ^{1}H, ^{13}C, and ^{15}N chemical-shift referencing. Indirect referencing to ^{1}H and use of Ξ ratios is one approach (*10*), but is unsuitable on older spectrometers. A second method, applicable to all instruments, would be to design a suitable universal internal reference. Here, we propose such a standard: *N, N', N''*-trimethyl(2-trimethylsilyl)ethylammonium iodide, TMS-A.

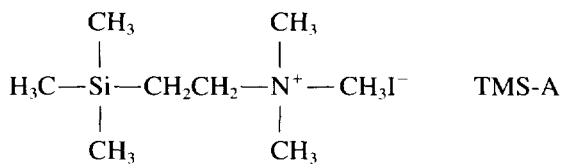
The two most common standards for work in H_2O (where use of TMS itself is impractical) are TSP (trimethylsilylpropionic acid, sodium salt) and DSS (dimethylsilapentanesulfonic acid, sodium salt), shown below:



TSP



DSS



TMS-A

Both are commonly used as internal standards. However, neither has a nitrogen functionality and TSP has a large pH dependence to its shift, due to titration of the carboxyl group (*9, 10*). We have therefore designed and synthesized an alternative TMS derivative, the ammonium salt shown above. TMS-A has the same trimethylsilylethyl fragment as do TSP and DSS, providing for ^{1}H, ^{13}C (and ^{29}Si) referencing in a generally uncluttered region of the spectrum (upfield from most protein resonances), and the quaternary nitrogen site group provides a suitable ^{15}N internal reference standard which is neither pH nor temperature sensitive, unlike NH_4^+ or external $NH_{3(\ell)}$.

We prepared TMS-A in three steps using the methods of Urayama *et al.* (*11*), Lutsenko *et al.* (*12*), and Sato *et al.* (*13*) as follows:

$$(CH_3)_3SiCl + CH_3CON(CH_3)_2 \xrightarrow[THF]{LDA} (CH_3)_3SiCH_2CON(CH_3)_2 \quad [1]$$

$$(CH_3)_3SiCH_2CON(CH_3)_2 \xrightarrow[Et_2O,\ \Delta]{LiAlH_4} (CH_3)_3SiCH_2CH_2N(CH_3)_2 \quad [2]$$

* This work was supported in part by the United States Public Health Service (NIH Grants HL-19481 and GM-50694). Partial funding was also provided by the Colgate-Palmolive Corporation (D.D.L.).

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1064-1866/95 $12.00

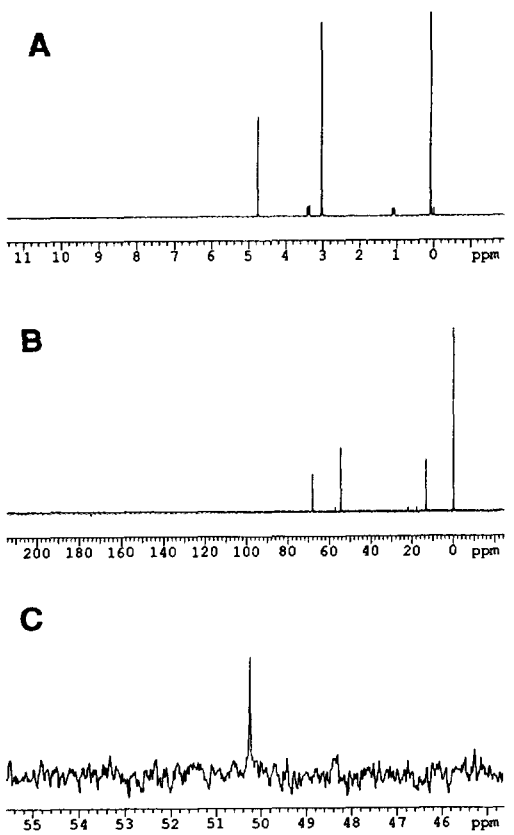

FIG. 1. NMR spectra of TMS-A obtained at 7 T, 25°C, in 99.8% D_2O. (A) ^1H spectrum of TMS-A (~5 mg/ml) referenced to an internal DSS standard (10 m*M*). The spectrum was acquired using a sweep width of 4000 Hz with a spectral resolution of 0.1 Hz/pt. (B) ^{13}C spectrum of TMS-A (~5 mg/ml) referenced to an internal DSS standard (10 m*M*) obtained using a sweep width of 18 kHz with WALTZ-16 decoupling. Resolution was 0.5 Hz/pt. (C) ^{15}N spectrum of TMS-A (~50 mg/ml) referenced externally to a sealed liquid ammonia sample. The spectrum was obtained using a sweep width of 15 kHz with WALTZ-16 decoupling during the acquisition period only; resolution was 0.5 Hz/pt.

$$(CH_3)_3SiCH_2CH_2N(CH_3)_2 + CH_3I \xrightarrow[\text{Acetone}]{\Delta} [(CH_3)_3SiCH_2CH_2N(CH_3)_3]I. \quad [3]$$

If ^{15}N labeling is desired, *N,N'*-dimethylacet[^{15}N]amide can be prepared according to the method of Coppinger (*14*), from ^{15}N-DMF and acetic anhydride.

^1H, ^{13}C, and ^{15}N spectra of TMS-A were recorded and are shown in Fig. 1. All measurements were performed in aqueous solution on a 300 MHz ^1H instrument and were referenced either internally to DSS (for ^1H and ^{13}C) or externally to a sealed sample of liquid ammonia (^{15}N). To test the stability of TMS-A under a wide variety of conditions, ^1H NMR spectra were obtained from pH 3.0 to 8.3 and at temperatures from 25 to 55°C. In each case, the chemical shift of the TMS-A trimethylsilyl protons remained constant, 0.081 ppm downfield from internal DSS. The ^{13}C chemical shifts were also temperature and pH independent, the observed shift being 0.23 ppm upfield from internal DSS. The ^{15}N resonance was observed to be 50.25 ppm upfield from $NH_{3(\ell)}$, at 25°C. The temperature dependence of the ^{15}N shift between 25 and 45°C was 7 ppb $°C^{-1}$, with a 1°C rise in temperature leading to a downfield shift of 7 ppb. The resonance frequency was also essentially pH independent between pH 4.0 and 10.5.

Due to its unique ability to provide chemically stable, pH- and temperature-independent chemical shifts for ^1H, ^{13}C, and ^{15}N NMR, and its relatively simple synthesis, TMS-A is a logical choice for a universal internal chemical-shift standard for multidimensional protein NMR studies. TMS-A also has the desirable property of being soluble in many commonly used NMR solvents, including ethanol, chloroform, DMF, acetone, and DMSO, making it a good candidate for an internal standard for the referencing of organic and inorganic compounds as well. Adopting such a standard would provide for much greater consistency in ^1H, ^{13}C, ^{15}N, and potentially ^{29}Si chemical-shift referencing, permitting the extraction of much more accurate structural information from NMR studies (*7, 8*).

ACKNOWLEDGMENT

We thank Professor T. B. Rauchfuss for providing advice and facilities.

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