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DSA: A New Internal Standard for NMR Studies in Aqueous Solution

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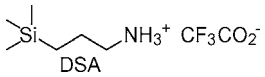
ABSTRACT



The widely used internal standard for NMR studies in aqueous solution DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate) can interact with cationic peptides, diminishing its value for such studies. This paper introduces DSA (4,4-dimethyl-4-silapentane-1-ammonium trifluoroacetate) as a new internal standard that does not suffer from this problem.

Sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS, **1**)[1] and sodium 3-trimethylsilyltetradeuteriopropionate (TSP, **2**)[2] are widely used as internal standards for NMR studies in aqueous solution. While using DSS as an internal standard for ^{1}H NMR studies of a variety of cationic peptides that fold into β-hairpin structures,[3,4] we observed variations in the chemical shift and broadness of the trimethylsilyl resonance of the DSS standard. These variations suggested that the DSS was interacting with some of the peptides and raised concerns about the wisdom of using an anionic compound bearing a hydrophobic group as an internal standard for cationic peptides containing hydrophobic aromatic residues. A search of the literature revealed similar observations and concerns in other systems.[5] As chemical shift studies have emerged as an important probe of peptide and protein structure,[6−8] we decided to address this problem by developing a new internal standard that does not interact as strongly with cationic peptides bearing hydrophobic residues. In this paper, we introduce 4,4-dimethyl-4-silapentane-1-ammonium trifluoroacetate (DSA, **3**) as a new internal standard for NMR studies in aqueous solution and compare it to DSS.

4,4-Dimethyl-4-silapentane-1-amine and its hydrochloride salt have been prepared previously by a number of methods, including the Gabriel amine synthesis[9] using phthalimide and commercially available (3-chloropropyl)trimethylsilane.[10] In the traditional Gabriel synthesis, phthalimide is alkylated with an alkyl halide and the resulting alkylated phthalimide is treated with hydrazine to liberate the amine. We found liberation of the amine to proceed more smoothly using the sodium borohydride reduction protocol of Ganem and coworkers.[11] Thus, we have prepared DSA in both gram and

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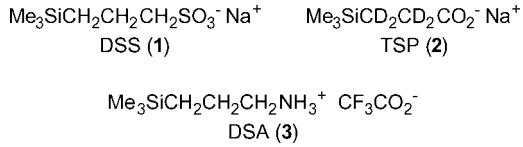


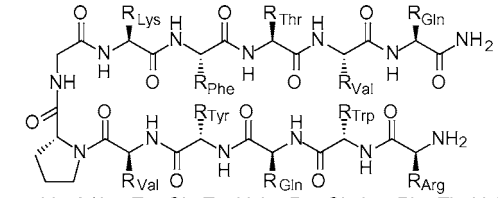
Figure 1. Internal standards for aqueous NMR studies.

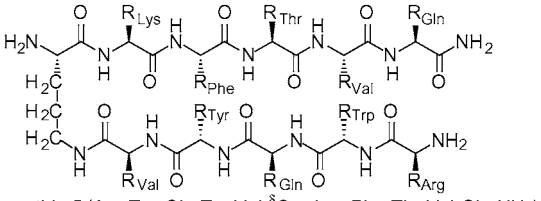
multigram quantities, in 77% overall yield, by alkylating phthalimide with (3-chloropropyl)trimethylsilane using K_2CO_3 in hot DMF, reduction with sodium borohydride, sequential acid and base extractions, acidification with trifluoroacetic acid (TFA), and recrystallization from toluene.

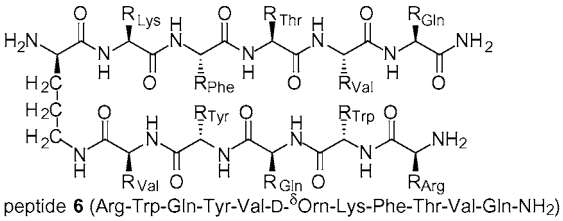
Like DSS, DSA is a free-flowing crystalline solid that is not visibly hygroscopic, even at high humidity. Although we had initially envisioned developing 4,4-dimethyl-4-silapentane-1-ammonium chloride as an internal standard, instead of the trifluoroacetate, the former compound proved to be deliquescent and inconvenient to handle. 4,4-Dimethyl-4-silapentane-1-amine is toxic and is a monoamine oxidase inactivator.[12] Although DSA should be treated with respect, the normal precautions (avoiding ingestion, skin contact, eye contact, etc.) should suffice for the submillimolar-concentration solutions typically used for NMR studies.

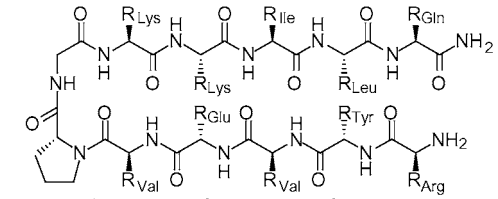
The ^{1}H NMR chemical shift of the trimethylsilyl group of DSA is the same as that of DSS in D_2O. At 298 K, we measure the DSA peak to be 4.761 ppm upfield of HOD and that of DSS to be 4.762 ppm. At temperatures ranging from 278 to 318 K, and at pHs ranging from 2 to 10, there are no significant differences ($<$0.005 ppm) in the positions of the trimethylsilyl resonances of the two compounds. The trimethylsilyl resonances are coincident in mixtures of DSA and DSS.

To evaluate the DSA, we studied its interaction with cationic peptides **4**−**9** (as the TFA salts), which are of interest for their potential to adopt β-hairpin structures. Peptide **4**, which was developed by Gellman and co-workers,[4] contains a β-turn based upon D-Pro-Gly and a hydrophobic cluster consisting of Trp, Tyr, Phe, and Val. Peptide **5** is an analogue in which the D-Pro-Gly turn is replaced with a turn based upon $^{\delta}$Orn, which was discovered in our laboratories.[3a] Both peptides **4** and **5** adopt β-hairpin structures. Peptide **6** contains D-$^{\delta}$Orn, in place of $^{\delta}$Orn, and does not fold into a β-hairpin structure.[3a] Peptide **7** was also developed by Gellman and co-workers[7c,13] and contains a β-turn based upon D-Pro-Gly but lacks the heavily aromatic hydrophobic cluster of the preceding peptides. Peptide **8** is an analogue of **7** that contains $^{\delta}$Orn in place of D-Pro-Gly but does not appear to fold into a β-hairpin structure.[14] Peptide **9** contains the $^{\delta}$Orn


peptide **4** (Arg-Trp-Gln-Tyr-Val-D-Pro-Gly-Lys-Phe-Thr-Val-Gln-NH_2)


peptide **5** (Arg-Trp-Gln-Tyr-Val-$^{\delta}$Orn-Lys-Phe-Thr-Val-Gln-NH_2)


peptide **6** (Arg-Trp-Gln-Tyr-Val-D-$^{\delta}$Orn-Lys-Phe-Thr-Val-Gln-NH_2)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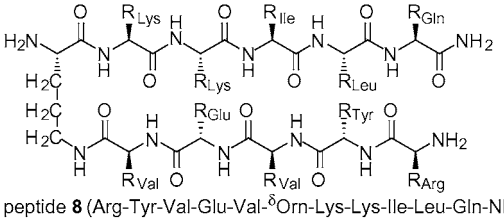
peptide **8** (Arg-Tyr-Val-Glu-Val-$^{\delta}$Orn-Lys-Lys-Ile-Leu-Gln-NH_2)

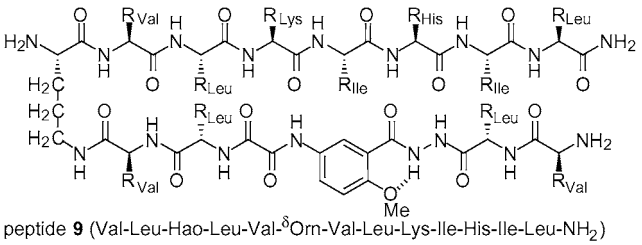
peptide **9** (Val-Leu-Hao-Leu-Val-$^{\delta}$Orn-Val-Leu-Lys-Ile-His-Ile-Leu-NH_2)

Figure 2. Peptides used in studies (as TFA salts).

turn, the unnatural amino acid *Hao*, which mimics a peptide β-strand,[15] and other hydrophobic residues. This peptide folds into a β-hairpin structure and will be described in detail in a future publication.[3b]

DSA and DSS were compared by measuring the chemical shift of the HOD resonance relative to that of DSA or DSS (set to 0.00 ppm) in the presence of millimolar concentrations of peptides **4**−**9** (Figure 3). Peptides **4** and **5** produced small but significant (0.01−0.04 ppm) downfield shifting of the

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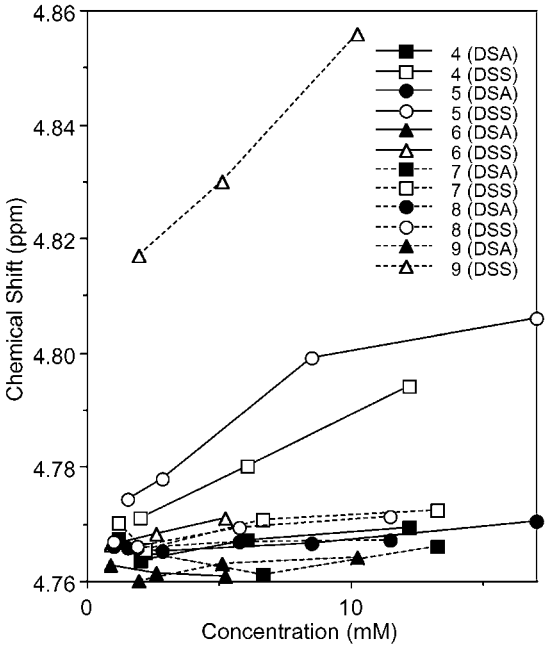

Figure 3. Chemical shift of the HOD resonance relative to that of DSA or DSS (set to 0.00 ppm) as a function of the concentration of added peptides **4**−**9**. Studies were performed on a 500 MHz ^{1}H NMR spectrometer in D_2O at 298 K with 0.1 mM DSA or DSS.

HOD resonance with DSS.[16] With DSA, these peptides caused little or no shifting (≤0.01 ppm). Peptides **6**−**8** produced no significant shifting with either DSA or DSS. In the presence of peptide **9**, the trimethylsilyl resonance of DSS is broad and indistinct. Although accurate referencing is not possible, peptide **9** appears to produce large (almost 0.1 ppm) downfield shifting with DSS. In contrast, the DSA peak is sharp, and there is no significant shifting (<0.01 ppm).

The peptides that fold and have multiple aromatic residues appear to be most sensitive to interaction with DSS. In such peptides, the cationic groups may interact with the anionic sulfonate group of DSS, while the aromatic residues may

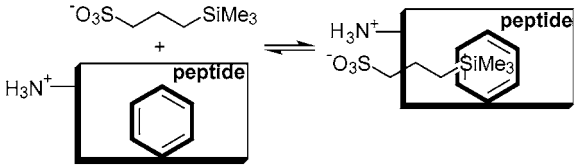

Figure 4. Model of interaction of DSS with cationic peptides containing aromatic residues.

interact with the hydrophobic trimethylsilyl group and shift the trimethylsilyl resonance upfield by magnetic anisotropy effects (Figure 4). When the trimethylsilyl resonance is used as a reference peak and set to 0.00 ppm, the water resonance and all of the peptide resonances are shifted downfield. It should be noted that the peptide typically is present in a large molar excess relative to the DSS and that the weak association between the peptide and DSS typically is dynamic on the NMR time scale. Thus, the peptide shifts the DSS, while the DSS has relatively little effect upon the peptide, which is largely unassociated due to its large molar excess.

DSA is superior to DSS as a reference compound for ^{1}H NMR studies of cationic peptides in aqueous solution, because it is less prone to interact with these peptides.[17] Because such peptides are currently the subject of widespread study and accurate measurement of chemical shifts is important in determining their folding properties, we anticipate that DSA will be of use to many other researchers.[6−8] We have prepared a supply of more than 15 g of DSA and are eager to share it (gratis) with others who will use it in their research. Requests for a sample should be sent by *principal investigators* at academic, industrial, or government laboratories to the corresponding author (J.S.N.).

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Supporting Information Available: Synthetic procedures for the preparation of DSA (**3**) and a ^{1}H NMR spectrum of DSA. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) We thank Professor Sam Gellman and Nick Fisk for initially preparing this peptide and bringing this finding to our attention.

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(16) These findings do not affect the conclusions of the DSS-based studies of peptides described in refs 3a and 4. At the peptide concentrations used for these studies, the resulting errors of ca. 0.01−0.02 ppm in chemical shift are not significant in comparison to the shift differences that are being measured.

(17) DSA should also be superior to TSP in these applications, as TSP should interact with peptides in a similar fashion and is sensitive to variations in pH. For details, see refs 8a and 8b.