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^{29}Si NMR Spectra of Trimethylsilyl and *tert*-Butyldimethylsilyl Derivatives of Purines and Pyrimidines

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ABSTRACT: Trimethylsilyl and *tert*-butyldimethylsilyl derivatives of naturally occurring purines and pyrimidines and also other closely related model compounds were prepared and their ^{29}Si NMR spectra measured. Only the chemical shifts of the Si—NH— moiety could be assigned experimentally (i.e. exactly); the chemical shifts of Si—O— and Si—N— fragments could be assigned only on the basis of chemical shift correlations. The Si—N— lines are surprisingly narrow in all derivatives of nucleic bases studied. The lines are narrow because of the 'self decoupling' of fast relaxing ^{14}N nuclei. The values of ^{29}Si, ^{15}N coupling constants could not be reliably determined (by the HEED–INEPT method), nor could the Si—N lines be completely assigned in these compounds. Measurement of ^{29}Si NMR spectra is shown to be a sensitive and fast method for checking completeness of silylation of nucleobases or their analogues. Assignment difficulties limit other applications. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{29}Si NMR; trimethylsilyl derivatives; *tert*-butyldimethylsilyl derivatives; INEPT; HEED–INEPT; purines; pyrimidines

INTRODUCTION

The reaction of silylated pyrimidine and purine bases with peracetylated sugars in the presence of Lewis acids is the standard procedure for the synthesis of nucleoside analogues.[1–3] The silyl derivatives used in this reaction are the labile and volatile trimethylsilyl (TMS) ethers of the nucleobases. *tert*-Butyldimethylsilyl (TBDMS) ethers of nucleoside bases are more stable but, hitherto, have not been used in nucleoside chemistry and no information on their structural characteristics is available. The TBDMS group, however, is extensively used as a hydroxyl protecting group in all branches of organic chemistry.[4,5] Also, oligonucleotides containing an internucleotide silyl linkage have been synthesized[6,7] as interesting neutral analogues of natural oligonucleotides and these compounds were recently studied by means of ^{1}H, ^{29}Si long-range heteronuclear multiple quantum spectroscopy.[8] Despite this, ^{29}Si NMR data for these compounds are not yet available in the literature. We are currently trying to fill this gap and find out if such data could be of any value to synthetic organic chemists.

EXPERIMENTAL

TMS derivatives of heterocyclic bases were prepared by stirring the neat base with a 1.3–1.5-fold molar excess of a chosen silylating reagent in a sealed flask at 100 °C for 3 h, followed by the removal of the unreacted reagent and other side-products under reduced pressure. For purine bases bis(trimethylsilyl)acetamide (BSA) was used as the silylating agent, whereas pyrimidine bases were silylated using volatile trimethylsilyldiethylamine (TMSDEA) to allow separation from low-boiling products by distillation.

An analogous reaction procedure was employed for the preparation of TBDMS derivatives using *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (TBDMS-FA) as a silylating reagent.[9] Thus, *ca.* 0.5 g of neat base was stirred in a sealed flask at a comparably high temperature (120–160 °C). Whereas a 1.3–2-fold excess of reagent and shorter reaction time (4 h) were sufficient for pyrimidine bases, the use of a large excess (20-fold) of TBDMS-FA and a longer reaction time (48 h) proved to be necessary in the case of less reactive purine bases. Pure products were obtained as a non-volatile fraction after evaporating the volatiles.

The NMR spectra were measured in dry chloroform-*d* solutions containing 1% (v/v) of hexamethyldisilane (HMDSS) as a secondary reference. A high sample concentration (*ca.* 50%, v/v) was used in HEED–INEPT measurements of coupling constants $J(^{29}\text{Si}, ^{15}\text{N})$. The reported chemical shifts were obtained from diluted solutions. The concentration of the sample was reduced until the ^{13}C chemical shift of HMDSS was $\delta = -2.48 \pm 0.02$, relative to the central line of the solvent at 76.99 pm (see Ref. 10 for the details of this standard procedure).

All the NMR spectral measurements were performed on Varian spectrometers. A Model VXR-400 was used for ^{14}N NMR measurements (at 28.898 MHz) with a 10 mm broadband probe which was slightly adapted to

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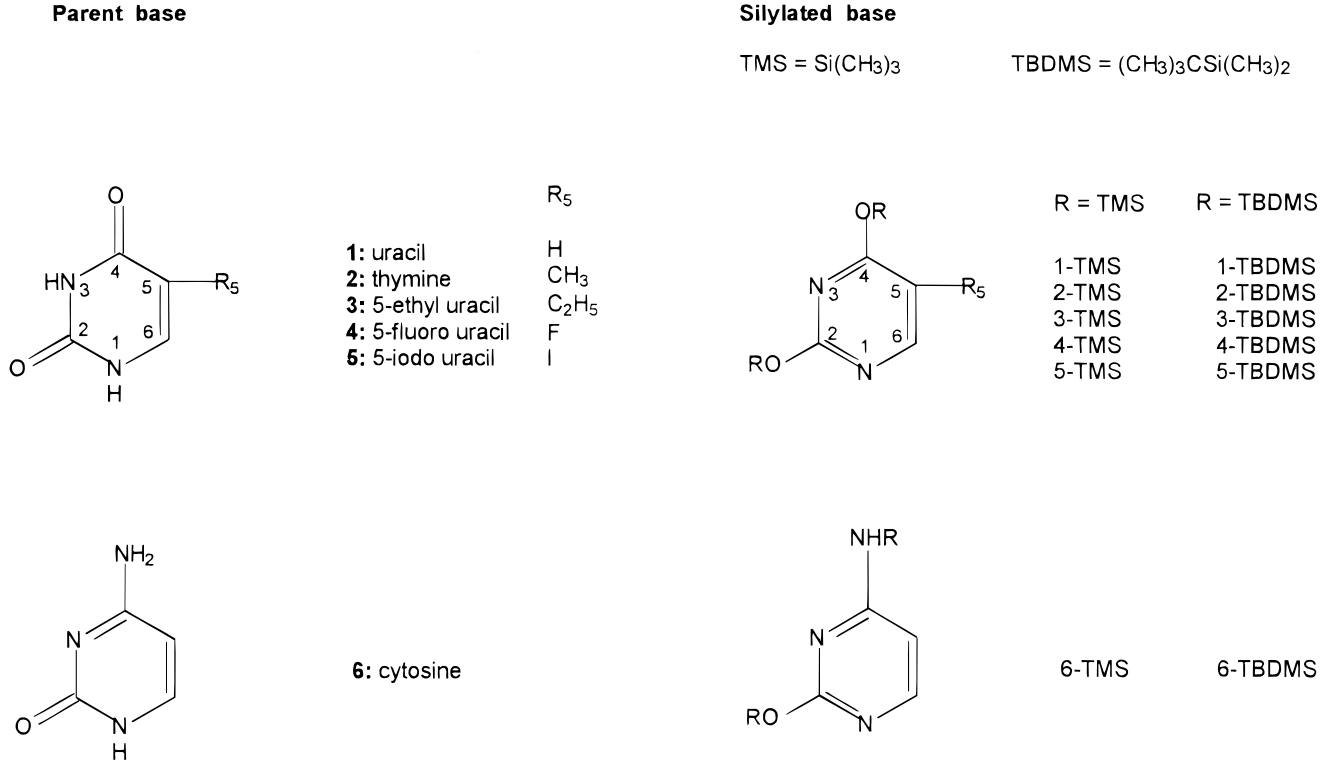
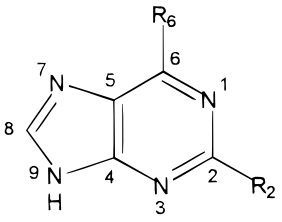
accept 5 mm sample tubes. Other measurements were made on a UNITY-200 spectrometer (operating at 50.3 MHz for ^{13}C and at 39.7 MHz for ^{29}Si NMR), using standard software (APT and INEPT pulse sequences). The spectra were recorded in the temperature range 22–24 °C. The ^{29}Si NMR spectra were measured by INEPT with the pulse sequence optimized[10] for TMS derivatives, i.e. for coupling to nine protons and a coupling constant of 6.5 Hz. The signal loss in the case of TBDMS derivatives was negligible.[11] Acquisition (1.0 s) was followed by a relaxation delay of 5 s. During the acquisition period WALTZ decoupling was used and FID data (8K) were sampled for the spectral width of 4000 Hz. Zero-filling to 32K and a mild exponential broadening were used in the data processing. The ^{29}Si $\pi/2$ pulses were at the maximum 20 μs long whereas for ^{1}H they were 11.5 μs in a 5 mm switchable probe. The ^{29}Si spectra were referenced to the line of HMDSS at $\delta = -19.79$. The ^{13}C NMR spectra were measured using a spectral width of 16 kHz. WALTZ decoupling was applied both during acquisition (1 s) and relaxation delay (2–5 s). Zero-filling to 64K and 1–3 Hz line broadening were used in data processing. The ^{29}Si lines were assigned by selective INEPT and selective decoupling.[12] Long-range ^{29}Si, ^{1}H coupling constants were determined either from 1D spectra or selective 2D *J*-resolved spectra.[12] The methods described in the Results and Discussion section (HEED–INEPT) were derived from the standard INEPT and were run on the UNITY-200 spectrometer.

The isolated compounds and their carbon atoms are numbered according to Scheme 1. The compounds were identified and checked by ^{13}C and ^{1}H NMR spectroscopy and the chemical shifts are given for convenience in Tables 1 and 2 (^{1}H NMR spectra were analysed only to the first order). Although the main results, ^{29}Si chemical shifts, are given later in Table 3, some additional details are briefly mentioned here.

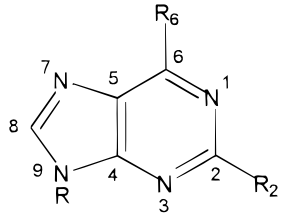
No visible coupling of ^{29}Si to any other proton but CH_3 protons is visible in the ^{29}Si NMR spectrum of **1-TMS**. In **2-TMS**, the ^{29}Si line at $\delta = 22.82$ exhibits a coupling of $J = 0.5$ Hz to a skeletal proton and the CH_3 protons on this silicon atom are at $\delta = 0.382$; the ^{29}Si line at $\delta = 21.12$ has the corresponding CH_3 proton signal at $\delta = 0.362$. In **6-TMS**, the ^{29}Si line at $\delta = 6.11$ exhibits a coupling $J = 2.4$ Hz to the NH proton and CH_3 protons of this silyl group resonate at $\delta = 0.313$; the ^{29}Si line at $\delta = 20.94$ has the corresponding CH_3 proton signal at $\delta = 0.360$. In **7-TMS**, the ^{29}Si line at $\delta = 14.77$ exhibits a coupling $J = 0.6$ Hz to a single proton and the corresponding CH_3 protons are at $\delta = 0.613$. INEPT with selective decoupling proves (see Fig. 2) that the ^{29}Si line at $\delta = 6.88$ is due to the Si—NH— moiety, $^{2}J(^{29}Si, ^{1}H) = 3.4$ Hz, and CH_3 protons of this group are at $\delta = 0.382$. In **7-TBDMS**, the ^{29}Si INEPT experiment with selective decoupling proves that the line at $\delta = 11.62$ is due to the Si—NH moiety with a coupling constant $^{2}J(^{29}Si, ^{1}H) = 2.6$ Hz, and ^{14}N NMR shows three lines at 60, 138 and 220 ppm relative to external NH_4Cl, with linewidths of 1000, 550 and 1000 Hz, respectively. In **11-TMS**, the ^{29}Si lines at $\delta = 23.65$, 13.41 and 3.39 have CH_3 proton signals at $\delta = 0.432$, 0.567 and 0.317, respectively. A coupling constant $J(^{29}Si, ^{15}N) = 13.5$ Hz was found in **13-TMS**. Similar coupling, $J(^{29}Si, ^{15}N) = 11.8$ Hz, was found in **13-TBDMS** in which the ^{14}N line occurs at $\delta = 133.8$ relative to external NH_4Cl with a linewidth of 153 Hz. Couplings of $J(^{29}Si, ^{15}N) = 12.2$ Hz and 10.2



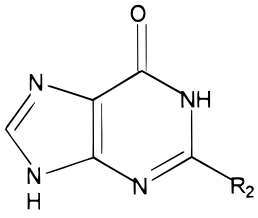
Scheme 1



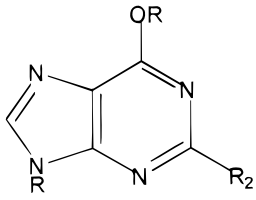
	R_2	R_6
7: adenine, R = H	H	NHR
8: 6-chloropurine	H	Cl
9: 2,6-dichloropurine	Cl	Cl



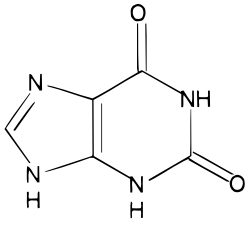
7-TMS 7-TBDMS
8-TMS 8-TBDMS
9-TMS 9-TBDMS



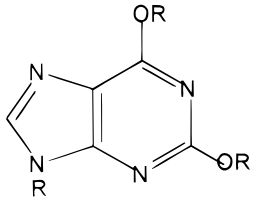
	R_2
10: hypoxanthine	H
11: guanine, R = H	NHR



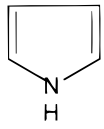
10-TMS 10-TBDMS
11-TMS 11-TBDMS



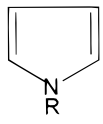
12: xanthine



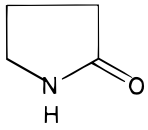
12-TMS 12-TBDMS



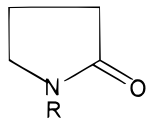
13: pyrrole



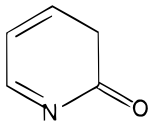
13-TMS 13-TBDMS



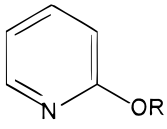
14: 2-pyrrolidinone



14-TMS 14-TBDMS



15: 2-pyridone



15-TMS 15-TBDMS

Scheme 1 continued

Hz were found in the spectra of **14-TMS** and **14-TBDMS**, respectively.

RESULTS AND DISCUSSION

The experimental results (^{29}Si chemical shifts and linewidths) are summarized in Table 3 for all the silylated bases and model compounds studied. The structural formulae and atom numbering in the parent bases are shown on Scheme 1. In all cases we observed the correct number of lines in the ^{29}Si NMR spectrum corresponding to the fully silylated product (i.e. all acidic protons

Table 1. ^{13}C chemical shifts for silylated bases[a]

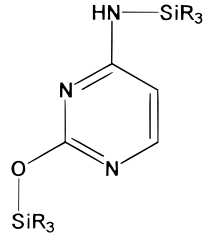
Compound[b]	C-2	C-4	C-5	C-6	C-8	CH_3-Si	CH_3-(C)	C
1-TMS	163.31[c]	169.69[c]	159.85[d]	104.25[d]	—	0.35, 0.31	—	—
1-TBDMS	170.25[c]	163.68[c]	159.88[d]	104.33[d]	—	−4.47, −4.56	25.57, 25.49	17.87, 17.87
2-TMS[e]	161.75	168.11	112.58	158.86[d]	—	0.37, 0.32	—	—
2-TBDMS[f]	162.06	168.29	112.72	158.82	—	−4.50, −4.50	25.60, 25.58	17.98, 17.90
3-TMS[g]	161.70	167.87	118.37	157.95	—	0.36, 0.34	—	—
3-TBDMS[h]	161.94	168.07	118.49	157.93	—	−4.48, −4.51	25.58, 25.58	17.94, 17.88
4-TMS	158.11	158.18	144.17	144.78	—	0.26, 0.19	—	—
4-TBDMS	158.44	158.30	144.35	146.76	—	−4.52, −4.61	25.56, 25.39	17.99, 17.89
5-TMS	163.00	167.54	72.59	165.99	—	0.19, 0.19	—	—
5-TBDMS	163.62	167.92	72.83	165.94	—	−4.61, −4.67	25.49, 25.49	18.04, 17.89
6-TMS	166.63[c]	163.52[c]	157.65[d]	101.15[d]	—	0.39, −0.24	—	—
6-TBDMS	167.03[c]	163.80[c]	157.53[d]	101.02[d]	—	−4.36, −4.61	26.10, 25.79	17.95, 17.46
7-TMS	152.47[c]	157.72[d]	154.80[d]	138.00[d]	142.03[c]	−0.11, −0.73	—	—
7-TBDMS	152.36	155.05	122.81	157.97	142.78	−4.45, −4.94	26.45, 26.02	18.29, 17.49
8-TMS	151.29[d]	156.42[c]	150.09[c]	133.01[c]	147.07[d]	−1.12	—	—
8-TBDMS	151.66[d]	157.15[c]	150.66[c]	133.15[c]	148.08[d]	−5.00	25.91	18.38
9-TMS	158.07[c]	152.62[c]	151.19[c]	132.44[c]	147.99	−0.82	—	
9-TBDMS	158.40[c]	152.51[c]	151.32[c]	132.29[c]	148.62	−5.04	25.87	18.31
10-TMS	151.15[d]	150.18[c]	157.52[c]	124.00[c]	143.65[d]	0.01, −1.31	—	—
10-TBDMS	151.67[d]	159.91[c]	158.36[c]	124.48[c]	145.08[d]	−4.16, −4.93	26.01, 25.86	18.35, 18.18
11-TMS	160.01[c]	159.78[c]	159.67[c]	118.43[c]	140.99	0.67, −0.17, −0.71	—	—
11-TBDMS	160.55[c]	160.14[c]	159.85[c]	118.53[c]	142.24	−3.99, −4.68, 4.68	26.25, 26.07, 25.90	18.32, 18.21, 16.89
12-TMS	160.53[c]	159.30[c]	158.98[c]	120.70[c]	142.64	0.60, 0.43, −0.68	—	—
12-TBDMS	160.70[c]	159.67[c]	158.80[c]	120.35[c]	143.56	−4.19, −4.73, −4.98	25.91, 25.84, 25.42	18.20, 19.10, 17.87
13-TMS[i]	122.91[j]	—	—	—	—	−0.32	—	—
13-TBDMS[k]	123.92[j]	—	—	—	—	1.93	25.84	18.17
14-TMS[l]	183.32	32.74[c]	46.97	—	—	1.91	—	—
14-TBDMS[m]	183.25	32.88[c]	47.97	—	—	−5.91	26.61	19.39
15-TMS[n]	162.54	138.84	116.71	147.25	—	0.45	—	—
15-TBDMS[o]	162.76	138.75	116.72	147.30	—	−4.28	25.81	18.02

[a] Chemical shifts on the δ scale, accurate to ± 0.02 ppm.
[b] For the compound and carbon atom numbering, see Scheme 1.
[c,d] Assignments can be interchanged.
[e] $\delta(CH_3) = 12.25$.
[f] $\delta(CH_3) = 12.45$.
[g] $\delta(CH_3) = 13.47$, $\delta(CH_2) = 20.35$.
[h] $\delta(CH_3) = 13.78$, $\delta(CH_2) = 20.38$.
[i] δ(C-3) = 110.72.
[j] A broad line.
[k] δ(C-3) = 110.30.
[l] δ(C-3) = 21.62[c].
[m] δ(C-3) = 21.90.
[n] δ(C-3) = 112.77.
[o] δ(C-3) = 113.01.

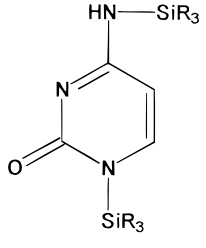
are replaced by silyl groups). Hence, for brevity, we refer here to the fully silylated product by the number of the parent compound (Scheme 1) to which we append either **-TMS** or **-TBDMS** to denote the type of silyl group. It is known from earlier MS studies[13] that the bases are silylated exclusively in enol forms if such can be formed. An example is given in Scheme 2 for cytosine derivatives. Of the two structures, only compounds with a structure like **6a** are formed.

Line assignment

It is relatively straightforward to establish which ^{29}Si line is due to an —Si—NH— moiety (which is present in the studied derivatives of cytosine, adenine and guanine). Silicon-29 NMR lines in that case are found in a distinct region of δ (between −3.0 and +8.0[14]) and, moreover, these lines can be assigned exactly by a number of different methods,[10,12] all based on the pres-



6a



6b

Scheme 2

Table 2. ^{1}H chemical shifts for silylated bases[a]

Compound[b]	HC-2	HC-5	HC-6	HC-8	CH_3-Si	CH_3C	NH
1-TMS	—	6.300[c]	8.196[c]	—	0.378, 0.378	—	—
1-TBDMS	—	6.297[d]	8.204[d]	—	0.355, 0.354	1.004, 0.978	—
2-TMS[e]	—	—	8.031	—	0.382, 0.362	—	—
2-TBDMS[f]	—	—	8.030	—	0.367, 0.332	0.999	—
3-TMS[g]	—	—	8.031	—	0.380, 0.366	—	—
3-TBDMS[h]	—	—	8.033	—	0.373, 0.337	1.001	—
4-TMS	—	—	8.078	—	0.421, 0.362	—	—
4-TBDMS	—	—	8.079	—	0.379, 0.326	1.005, 0.992	—
5-TMS	—	—	8.478	—	0.413, 0.370	—	—
5-TBDMS	—	—	8.470	—	0.382, 0.337	1.049, 0.994	—
6-TMS	—	7.967[i,j]	6.045[i,j]	—	0.360, 0.313	—	4.4[k]
6-TBDMS	—	7.964[j,l]	6.085[j,l]	—	0.334, 0.294	0.998, 0.963	4.4[k]
7-TMS	8.376[j]	—	—	7.724[j]	0.613, 0.382	—	5.25[k]
7-TBDMS	8.331[j]	—	—	7.725[j]	0.642, 0.379	1.024, 0.965	5.2[k]
8-TMS	8.741[j]	—	—	8.080[j]	0.668	—	—
8-TBDMS	8.732[j]	—	—	8.082[j]	0.705	0.974	—
9-TMS	—	—	—	8.089	0.673	—	—
9-TBDMS	—	—	—	8.055	0.695	0.981	—
10-TMS	8.463[j]	—	—	7.866[j]	0.629, 0.467	—	—
10-TBDMS	8.434[j]	—	—	7.875[j]	0.657, 0.441	1.071, 0.963	—
11-TMS	—	—	—	7.539	0.567, 0.432, 0.317	—	4.47[k]
11-TBDMS	—	—	—	7.572	0.610, 0.401, 0.317	1.051, 0.972, 0.947	4.3[k]
12-TMS	—	—	—	7.681	0.593, 0.459, 0.399	—	—
12-TBDMS	—	—	—	7.700	0.624, 0.421, 0.337	1.056, 0.996, 0.952	—
13-TMS[m]	6.81[j]	—	—	—	0.415	—	—
13-TBDMS[n]	6.79[j]	—	—	—	0.420	0.880	—
14-TMS[o]	—	3.4[j]	—	—	0.040	—	—
14-TBDMS[p]	—	3.393[q]	—	—	0.267	0.950	—
15-TMS[r]	—	8.10[j]	7.54[j]	—	0.349	—	—
15-TBDMS[s]	—	8.10[j]	7.54[j]	—	0.318	0.987	—

[a] Chemical shifts on the δ scale, accurate to $\pm$0.002 ppm, first-order analysis only.
[b] For the compound and carbon atom numbering, see Scheme 1.
[c] J = 5.6 Hz.
[d] J = 5.7 Hz.
[e] $\delta(CH_3)$ = 2.017.
[f] $\delta(CH_3)$ = 2.039.
[g] $\delta(CH_3)$ = 1.146, $\delta(CH_2)$ = 2.445, J = 7.4 Hz.
[h] $\delta(CH_3)$ = 1.155, $\delta(CH_2)$ = 2.467, J = 7.4 Hz.
[i] J = 5.9 Hz.
[j] Assignment can be interchanged.
[k] A broad line.
[l] J = 5.8 Hz.
[m] $\delta(CH)$ = 6.33[j].
[n] $\delta(CH)$ = 6.32[j].
[o] $\delta(CH)$ = 2.3[j] and 2.1[j].
[p] $\delta(CH_2$-3) = 2.338, J = 8.0 Hz, $\delta(CH_2$-4) = 2.027, J = 6.8 and 8.0 Hz.
[q] J = 6.8 Hz.
[r] $\delta(CH)$ = 6.83[j] and 6.66[j].
[s] $\delta(CH)$ = 6.83[j] and 6.68[j].

ence of the two-bond coupling, $^{2}J(^{29}Si, N, ^{1}H)$. The simplest method is probably INEPT with selective decoupling during acquisition. Figure 1 shows an example of its application. Clearly, the additional doublet structure of the anti-phase multiplet centered around 6.8, which disappears with selective decoupling of the NH proton, is due to spin–spin coupling with the NH proton.

When there are two or more silyl groups other than Si—NH groups in the molecule, we encounter a difficult assignment problem. To assign two Si—O or two Si—N lines exactly in the studied compounds, one must resort to ^{29}Si–^{13}C or ^{29}Si–^{15}N correlations.[15–17] Since the necessary hardware is not at our disposal, we offer only tentative assignments for these cases later.

Similarly, we were unable to differentiate experimentally the lines due to Si—N and Si—O moieties in the studied compounds. Usually, these lines could be distinguished according to their linewidths and/or by the presence (or absence) of ^{29}Si–^{15}N couplings. As the values in Table 1 indicate, the linewidths do not show much variation (except for pyrrole and 2-pyrrolidone derivatives); they are essentially determined by the acquisition time. Separate experiments using much

Table 3. ^{29}Si chemical shifts for silylated bases[a]

Base	TMS derivative		TBDMS derivative		Calculated		
	δ (ppm)	Linewidth (Hz)	δ (ppm)	Linewidth (Hz)	δ(TBDMS)[b] (ppm)	Δ(TBDMS)[c] (ppm)	Assignment[d]
Pyrimidines:							
Uracil	23.29	0.6	24.46	0.6	24.48	−0.02	Si—O—4
	21.88	0.6	23.07	0.6	23.08	−0.01	Si—O—2
Thymine	22.82	0.5	23.99	0.6	24.01	−0.02	Si—O—4
	21.12	0.5	22.30	0.5	22.33	−0.03	Si—O—2
5-Ethyluracil	22.73	0.5	24.01	0.3	23.92	0.09	Si—O—4
	21.10	0.5	22.23	0.3	22.31	−0.08	Si—O—2
5-Fluorouracil	26.52	0.7	27.29	0.4	27.67	−0.38	Si—O—4
	22.45	0.7	23.59	0.4	23.65	−0.06	Si—O—2
5-Iodouracil	25.75	0.7	26.96	0.4	26.91	0.05	Si—O—4
	23.13	0.7	24.10	0.4	24.32	−0.22	Si—O—2
Cytosine	20.96	0.7	22.66	0.6	22.17	0.49	Si—O
	6.11	0.8	10.32	0.6	7.47	2.85	Si—NH
Purines:							
Adenine	14.77	0.6	19.19	0.6	16.04	3.15	Si—N
	6.88	0.6	11.62	0.6	8.23	3.39	Si—NH
Guanine	23.65	0.7	24.93	0.7	24.83	0.10	Si—O
	13.41	0.7	17.99	0.7	14.70	3.29	Si—N
	3.39	0.7	8.06	0.7	4.78	3.28	Si—NH
Xanthine	24.81	0.4	26.34	0.3	25.98	0.36	Si—O
	20.82	0.4	21.73	0.3	22.03	−0.30	Si—O
	14.48	0.4	18.81	0.4	15.76	3.05	Si—N—9
6-Chloropurine	17.66	1.0	21.75	0.8	18.90	2.85	Si—N
2,6-Dichloropurine	18.81	0.4	22.72	0.8	20.04	2.68	Si—N
Hypoxanthine	25.27	0.3	26.41	0.4	26.44	−0.03	Si—O
	15.51	0.5	19.72	0.5	16.78	2.94	Si—N
Miscellaneous:							
Pyrrole	12.04	3.5	15.91	2.2	13.34	2.57	Si—N
2-Pyrrolidone	8.71	0.9	13.48	0.8	10.04	3.44	Si—N
2-Pyridone	20.18	0.2	21.86	0.4	21.40	0.46	Si—O

[a] Chemical shifts on the δ scale, accurate to $\pm$0.02 ppm. For additional data, see Experimental.
[b] Chemical shifts δ(TBDMS) calculated from correlation δ(TBDMS) = 1.422 + 0.9899 δ(TMS).
[c] The difference between the experimental and calculated chemical shifts in the TBDMS derivative, $\Delta = \delta$(TBDMS-experimental) − δ(TBDMS-calculated).
[d] Assignment to Si—O—, Si—N— or Si—NH— moieties; the number refers to the nearest atom of the base skeleton; for details, see text.

longer acquisition times (4–6 s) yielded lines accordingly narrower but again without significant differences between Si—O and Si—N lines. Using the results of the extensive work of Kupče and co-workers[17,18] and Wrackmeyer and co-workers[19,20] on these couplings, we attempted to employ their HEED method[21] to determine the coupling constants, $^1J(^{29}\text{Si}, ^{15}\text{N})$. To make the HEED method more robust, we modified it along the lines suggested for the off-resonance coherently decoupled INEPT experiment,[22] i.e. one pair of refocusing pulses was eliminated as unnecessary and the broadband decoupling was turned on after the usual INEPT refocusing time since the last $\pi/2$ pulse. The difference between the original and modified pulse sequence is apparent from Fig. 2. Both methods yielded the coupling constants for the model compounds (**13-TMS**, **13-TBDMS**, **14-TMS** and **14-TBDMS**; for the values found, see Experimental) which had broad ^{29}Si lines of Si—N moieties. In the case of silylated nucleic bases with narrow ^{29}Si line, as shown by the TBDMS derivative of adenine (**7-TBDMS**), even extremely long spin-echo times (e.g. $t = 10$ s) did not substantially reduce the intensity of the center lines (^{14}N) and no ^{15}N satellites could be identified with certainty; positive identification would require ^{15}N decoupling or a similar experiment. Measurement of ^{14}N NMR spectra of **7-TBDMS** and **13-TBDMS** showed, in agreement with the observed linewidths in the ^{29}Si NMR spectra and with the theory proposed,[21] that the ^{14}N NMR line of pyrrole is narrow and the line(s) of adenine are very broad. Obviously, fast relaxation of ^{14}N nuclei provides an effective decoupling of ^{29}Si NMR lines of Si—N— moieties in silylated nucleic bases, irrespective of values of the coupling constants $J(^{29}\text{Si}, ^{14}\text{N})$.

Correlations

Recently, we have reported on correlations holding between ^{29}Si chemical shifts in TMS and TBDMS

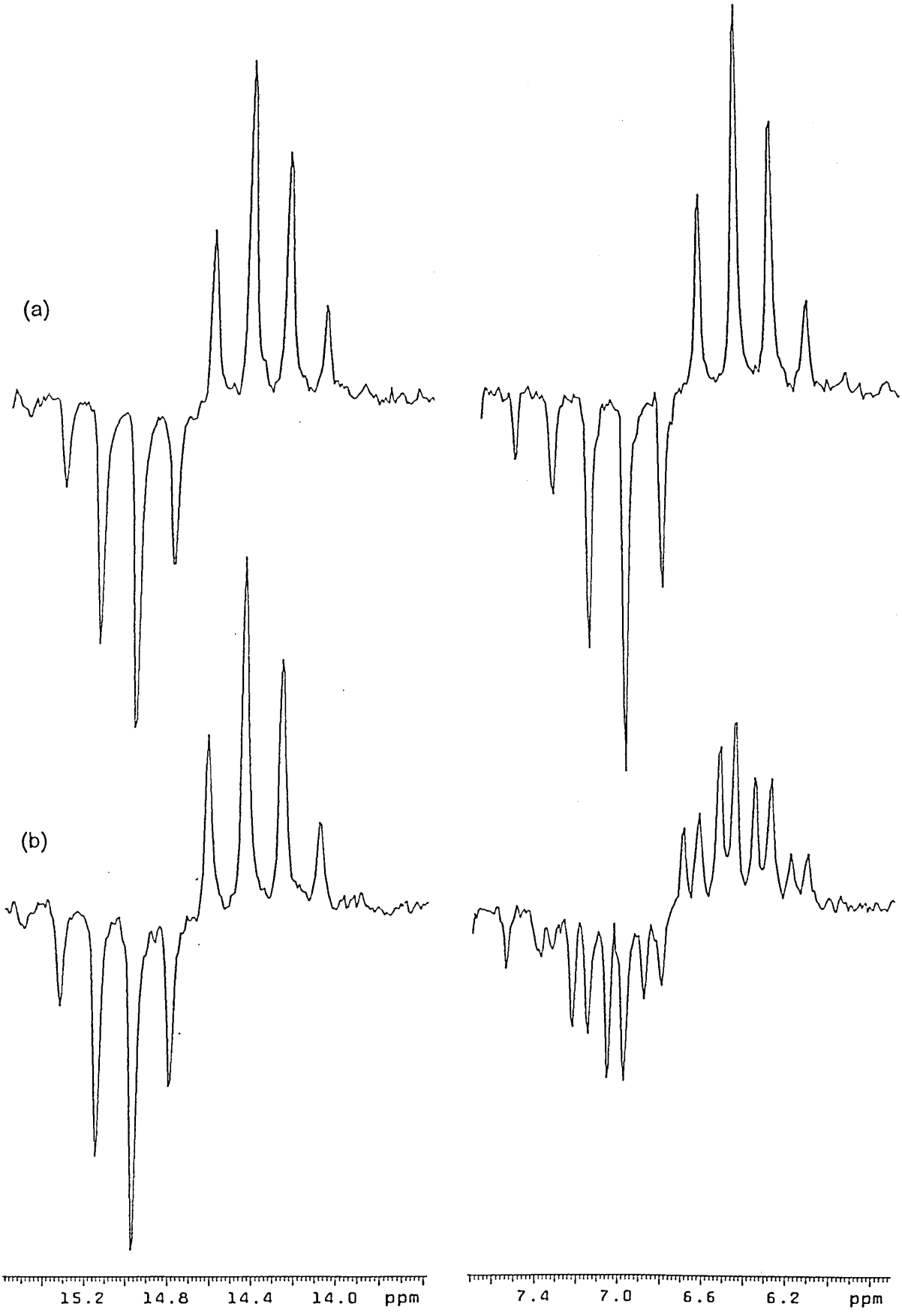


Figure 1. [29]Si NMR spectra of bis(trimethylsilyl)adenine measured (a) with selective decoupling of NH proton and (b) without decoupling during acquisition of [29]Si INEPT.

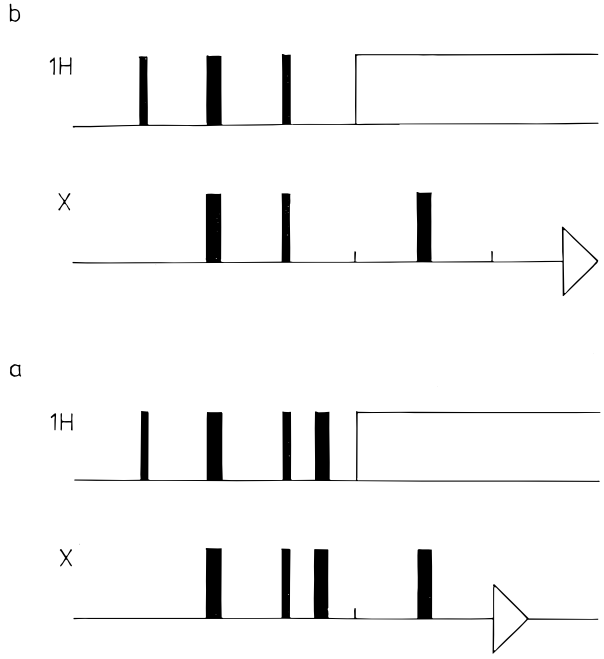


Figure 2. HEED–INEPT pulse sequences: (a) original pulse sequence[21] and (b) pulse sequence modified here. Schematic, only to show the simplification achieved by the modification. For timing, see Ref. 21; for phase cycling, see Ref. 22. Narrow filled rectangles denote $\pi/2$ pulses, broader filled rectangles denote π pulses, empty rectangles denote decoupling in the ^{1}H channel and triangles denote acquisition in the X channel, i.e. ^{29}Si here.

derivatives, δ(TMS) and δ(TBDMS), of amino acids[23] and alcohols.[24] So far, all the assigned chemical shifts of Si—O— moieties fit (see Table 1) the correlation described for alcohol derivatives very well (maximum deviation 0.5 ppm, but often less than 0.1 ppm). The lines assigned to Si—N— or Si—NH— moieties exhibit deviations in excess of 2 ppm. These large deviations are somewhat reduced (significantly reduced are those for Si—NH— moieties) if the correlation for amino acids[23] is employed instead.

In view of the enolic structures of the studied compounds, the observed small and large deviations are not surprising. The Si—O— moieties maintain the same local structure as in the model alcohol derivatives;[24] the Si—N— or Si—NH— are significantly different. In the Si—N— moieties studied here, the nitrogen is usually part of an aromatic ring and hence the reported shifts contain large paramagnetic contributions from ring currents. In the model amino acids there were no Si—NH— moieties attached to such aromatic rings.

Since these structural differences also apply to the compounds with ^{29}Si line not yet assigned to Si—O— or Si—N— moieties, we suggest the utilization of the deviations from the reported correlations for line assignment. Thus, in guanine and hypoxanthine derivatives the lines with deviations of 0.1 ppm or less are assigned to Si—O— and those with deviations of 2.9 ppm or more to Si—N— moieties. In xanthine derivatives the lines with deviations of less than 0.4 ppm are both assigned to Si—O— and those with deviation of 3.0 ppm to Si—N— moieties. Corollary supporting evidence for these assignments is provided by the chemical shift values; the Si—O— shifts thus assigned have larger values, both Si—O— and Si—N— values agree with the values found for other closely related compounds. Moreover, eleven pairs δ(TBDMS) and δ(TMS) values reported in Table 3 for Si—N— and Si—NH— moieties are also linearly correlated [$r = 0.969$, $\delta(\text{TBDMS}) = 0.995 + 1.2075 \cdot \delta(\text{TMS})$].

Comparing the chemical shift values in compounds with two Si—O— lines (**1–5**) with the chemical shift of the Si—O— line in cytosine (**6**), we tentatively assign the lower chemical shift values in **1–5** to the siloxy group attached to C-2. Variation in the value of this chemical shift in the series **1–5** [linear regression for $n = 5$ data points yielded $\delta(\text{TMS}) = 22.03 + 5.920\,57\sigma_p$ $r = 0.994$; $\delta(\text{TBDMS}) = 23.14 + 5.4869\sigma_p$), $r = 0.993$] reflects the well known sensitivity of ^{29}Si chemical shifts[25] to substituent effects. In this case it is the effect of the *para*-substituent (on C-5) which parallels that described for *para*-substituted trimethylsiloxybenzenes [$\delta(\text{TMS}) = 18.19 + 4.77\sigma_p$].[26]

Application

Since the measurements of ^{29}Si NMR spectra of TMS or TBDMS derivatives by INEPT or DEPT is very fast (*ca.* 20 min for a solution of 20 mg in 0.7 ml of solution) and since the chemical shifts measured under standard conditions[10] are very reproducible ($\pm$ 0.02 ppm), the measurement of ^{29}Si NMR spectra offers a simple and reliable way to check the completeness of silylation. The absence of a reliable routine method for line assignment in this case and the sensitivity of the ^{29}Si chemical shifts to structure make this measurement less suitable for the determination of the site of silylation in the case of incomplete silylation.

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