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Synthesis and Antileukemic Activities of Furanyl, Pyranyl, and Ribosyl Derivatives of 4-(3,3-Dimethyl-1-triazeno)imidazole-5-carboxamide and 3-(3,3-Dimethyl-1-triazeno)pyrazole-4-carboxamide

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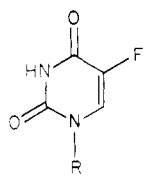
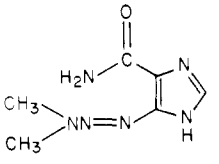
From the reaction of silylated 4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide (DTIC, **5**) and 3-(3,3-dimethyl-1-triazeno)pyrazole-4-carboxamide (DTPC, **9**) with 2-chlorotetrahydrofuran, we have isolated in both cases a single tetrahydrofuran-2-yl derivative. However, when silylated DTPC was reacted with 2-chlorotetrahydropyran, two tetrahydropyran-2-yl compounds were obtained, and these were shown to be positional isomers on the basis of ^{1}H NMR and UV data. These furanyl and pyranyl derivatives were tested for antileukemic activity (L-1210, in vivo), and the results were compared with the results obtained for the corresponding ribosyl derivatives of DTIC and DTPC.

The 1-(tetrahydrofuran-2-yl) derivative (**1**, Ftorafur) of





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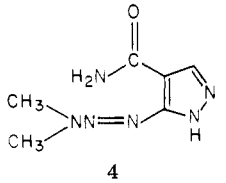
1, R = tetrahydrofuran-2-yl
2, R = H



4

5-fluorouracil (**2**, 5-FU) has aroused considerable interest as a potential replacement[1,2] for 5-fluorouracil in the treatment of cancer of the breast and the gastrointestinal tract. This interest in Ftorafur has been based on reports[3] that it displays a higher chemotherapeutic activity (twice that of 5-FU) and a lower toxicity (five to six times less than 5-FU) than that of the parent heterocyclic base. It has been suggested[4] that Ftorafur is a depot form of 5-FU and that its antitumor activity is due to released 5-FU. It has been demonstrated[5] that enzymatic cleavage of Ftorafur does take place in the liver, and of particular interest is the fact that the enzymatic reaction appears to be nonspecific, since both the *R* and *S* isomers of Ftorafur display[6] the same antibacterial and antitumor activities.

The interesting results with Ftorafur prompted us to prepare similar derivatives of the antitumor agent[7] 4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide (**3**, DTIC) and the isomeric compound 3-(3,3-dimethyl-1-triazeno)pyrazole-4-carboxamide (**4**, DTPC) on the premise that they might also serve as depot forms of these drugs. We were also interested in these derivatives, since the corresponding ribosides had been previously prepared[8,9] in our laboratory and were available for a direct comparison of chemotherapeutic activities.

Chemistry. We had previously found that a reaction of the silyl derivatives of DTIC and DTPC with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide (**6c**) afforded the corresponding β-D-ribofuranosyl derivatives **7c**, **8c**, **10c**, and **11c**, respectively. We have now extended this procedure to the synthesis of furanyl and pyranyl derivatives of these heterocyclic compounds (Scheme I).

A methylene chloride solution of 2-chlorotetrahydrofuran[10] (**6a**) was reacted with the trimethylsilyl derivative[8] (**5**) of DTIC. Our isolation procedure furnished a single crystalline product (28%). Elemental and ^{1}H NMR spectral analyses confirmed that the product was a tetrahydrofuran-2-yl derivative of DTIC. This product was assigned the structure 4-(3,3-dimethyl-1-triazeno)-1-(tetrahydrofuran-2-yl)imidazole-5-carboxamide (**7a**), since its UV spectrum was essentially identical with that

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observed[8] for the corresponding riboside (**7c**) of DTIC. The possibility that this product was the other N isomer (**8a**) of DTIC was eliminated, since we would have expected[8] the UV spectrum of the isomeric compound to display a UV absorption maximum at about 10-nm longer wavelength (methanol and pH 11) than what we actually observed.

A similar reaction between 2-chlorotetrahydrofuran (**6a**) and the crystalline trimethylsilyl derivative[9] (**9**) of DTPC and a similar isolation procedure also provided a single crystalline compound. This compound was characterized as 3-(3,3-dimethyl-1-triazeno)-1-(tetrahydrofuran-2-yl)-pyrazole-4-carboxamide (**10a**; 22.5% yield) on the basis of elemental analysis and spectral data. None of the other N-isomer (**11a**) was isolated from this reaction. However, when the trimethylsilyl derivative[9] of DTPC was reacted in the same manner with 2-chlorotetrahydropyran,[10] we isolated two crystalline compounds with nearly identical melting points, but a marked melting point depression was observed on admixture with each other. The elemental analyses indicated that both compounds possessed the same empirical formula. The ^{1}H NMR spectral data also indicated very similar structures for the two compounds. The major product (45% yield) was assigned the structure 3-(3,3-dimethyl-1-triazeno)-1-(tetrahydropyran-2-yl)-pyrazole-4-carboxamide (**10b**), and the minor product (14%) was assigned the structure 5-(3,3-dimethyl-1-triazeno)-1-(tetrahydropyran-2-yl)pyrazole-4-carboxamide (**11b**) on the basis of a comparison of UV spectra with spectral data reported[9] for the corresponding ribosyl derivative of DTPC. It is interesting to note that the isomer **10b**, which has the tetrahydropyran-2-yl group vicinal to H_5 of the pyrazole ring, shows a peak in the ^{1}H NMR spectrum of H_5 which is downfield ($\Delta\delta$ 0.49) compared to the signal observed for the pyrazole ring hydrogen (H_5) in the other isomer (**11b**). This phenomenon has been previously observed[9] for isomeric pairs of β-D-ribofuranosylpyrazoles. This observed effect is most likely due to a deshielding effect which the furanose and pyranose ring oxygens can exert on the pyrazole ring hydrogen. However, this deshielding effect can only occur when the furanose or pyranose groups are located in a position α to the pyrazole ring hydrogen.

Chemotherapeutic Activity. Table I summarizes the in vivo antileukemic activities (against L-1210) observed for the furanyl, pyranyl, and ribosyl derivatives of DTIC (**3**) and DTPC (**4**). Only one derivative of DTIC displayed significant antileukemic activity. This compound was 4-(3,3-dimethyl-1-triazeno)-1-(β-D-ribofuranosyl)imidazole-5-carboxamide (**7c**), which displayed a T/C of 157 at a dose of 400 mg/kg.

Interestingly, none of the pyranyl or ribosyl derivatives of DTPC demonstrated any significant antileukemic activity. On the other hand, the furanyl derivative 3-(3,3-dimethyl-1-triazeno)-1-(tetrahydrofuran-2-yl)pyrazole-4-carboxamide (**10a**) displayed significant activity, showing a T/C of 141 and 150 at a dose of 100 mg/kg which is nearly the same activity as that reported[12] for DTPC.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a Varian A-60 or a XL-100-12 spectrophotometer using dimethyl-d_6 sulfoxide as solvent and sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as an internal standard, unless otherwise noted, with the chemical shifts being expressed in δ units (parts per million) from the standard. UV spectra were determined on a Beckman DK-2 spectrophotometer. TLC were run using chromatographic grade silica gel (SilicAR 7GF) purchased from Mallinckrodt Co., and a short-range (254 nm) ultraviolet light was used for detection of compounds. Dry column chromatography was run using silica gel CC-7 (200–325 mesh) obtained from Mallinckrodt Co. Unless otherwise noted, concentrations were carried out in vacuo at 35 °C.

Table I. L-1210 in Vivo Testing Data for Certain Derivatives of DTIC and DTPC

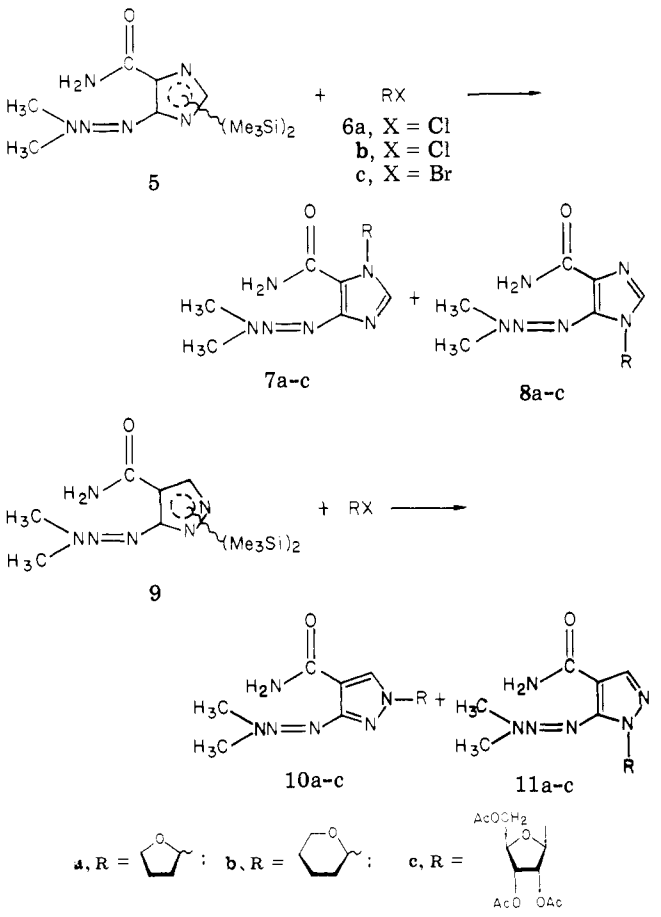
compd	dose,[a] mg/kg	surv	animal wt diff	surv, days	% T/C	test status	ref
DTIC (3)	180	6/6	−2.6		142		
	125	12/12	−2.1		147		11a
	120	17/18	−2.2		157		
	80	6/6	−2.0		147		
	53	6/6	−0.8		126		
8c	200	6/6	−0.9	8.7/9.1	95	22	
	100	6/6	−0.3	9.5/9.1	104	22	
7c	400	6/6	−2.2	15.7/10.0	157	23	
	200	6/6	−1.6	11.8/9.1	129	22	
	200	6/6	−2.7	11.8/9.5	124	23	
	100	6/6	−1.5	11.2/9.1	123	22	
7a	200	6/6	−4.3	11.8/10.2	115	22P	
	100	6/6	−1.9	12.8/10.2	125	22P	
DTPC (4)	180[c]	6/6	−4.9		134		12
	109[c]	6/6	−3.7		176		
	65[c]	6/6	−3.2		142		
10c	400[b]	6/6	−1.1	9.0/10.1	89	23	
	200[b]	6/6	−1.8	9.0/8.5	105	22	
	100[b]	6/6	−0.1	8.5/8.5	100	22	
	200[c]	6/6	2.8	9.8/9.1	107	23F	
10a	200	6/6	−3.7	12.5/9.3	134	22P	
	200	6/6	−5.0	13.5/9.3	142	22P	
	100	6/6	−3.2	14.3/9.5	150	22P	
	100	6/6	−2.3	13.2/9.3	141	22P	
	50	6/6	−1.8	12.0/9.3	129	22P	
10b	400[b]	5/6	−2.9	13.4/11.2	119	25F	
	200[b]	5/6	−1.0	12.2/11.2	108	25F	
	100[b]	6/6	−0.9	12.2/11.2	108	25F	
11c	200	6/6	−2.3	12.2/10.0	122	23F	
11b	400[b]	4/6	−3.5	13.8/11.2	123	25F	
	200[b]	6/6	−3.1	13.2/11.2	117	25F	
	100[b]	6/6	−2.2	12.0/11.2	107	25F	

[a] Unless otherwise noted: day 1 = first injection, five injections, 1-day intervals. [b] Day 1 = first injection, three injections, 4-day intervals. [c] Day 1 = first injection, nine injections, 1-day intervals.

4-(3,3-Dimethyl-1-triazeno)-1-(tetrahydrofuran-2-yl)-imidazole-5-carboxamide (7a). Dry 4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide[11b] (**3**; 3 g, 16.5 mmol) was suspended in dry methylene chloride (20 mL) and then 9 mL (37.5 mmol) of bis(trimethylsilyl)acetamide was added. The heterocycle slowly dissolved during 1 h of stirring at 25 °C. The methylene chloride was removed in vacuo, and the reaction mixture was heated (oil bath at 70 °C) in vacuo (1 Torr) to remove the last traces of volatile byproducts of the reaction. The residue was then dissolved in dry methylene chloride (30 mL), and the light yellow solution was cooled to −40 °C. Freshly prepared 2-chlorotetrahydrofuran[10] (2.54 g, 25 mmol) was added to the solution in one portion, and the reaction mixture was stored at 5 °C for 18 h. The reaction mixture was concentrated in vacuo, and the resulting residue was applied to the top of a glass column (4 × 25 cm) containing dry-packed SilicAR CC-7 (173 g). The column was eluted with acetone, and 43-mL fractions were collected. Fractions 5–23 were combined and concentrated to dryness in vacuo. The residue was recrystallized from methyl ethyl ketone (8 mL) to yield 1.17 g (28%) of **7a**, mp 169–170 °C dec. A small sample was recrystallized from methyl ethyl ketone for analysis: mp, 176–178 °C (bubbling); ^{1}H NMR (Me_2SO-d_6) δ 7.72 (s, 1 H, H_2), 7.44 (br s, 2 H, $CONH_2$), 6.70 (d, J = 2.8 Hz, $H_{1'}$), 3.53 and 3.22 [br s, 3 H, $N(CH_3)_2$]; UV λ_{max} ($\epsilon \times 10^{-3}$) (pH 1) 322 nm (ϵ 22.2), 275 (sh, 9.3), λ_{min} 246.5 (6.0); λ_{max} (CH_3OH) 322.5 (19.8), 237.3 (16.6), λ_{min} 259 (7.3), 222 (12.4); λ_{max} (pH 11) 325.5 (19.7), 239 (15.1), λ_{min} 260 (6.6). Anal. Calcd for $C_{10}H_{16}N_6O_2$: C, 47.57; H, 6.39; N, 33.31. Found: C, 47.87; H, 6.39; N, 33.57.

3-(3,3-Dimethyl-1-triazeno)-1-(tetrahydrofuran-2-yl)-pyrazole-4-carboxamide (10a). Dry 3-(3,3-dimethyl-1-triazeno)pyrazole-4-carboxamide (**4**; 5.0 g, 27.4 mmol) was tri-

Scheme I



methylsilylated as described previously.[9] The crystalline silyl derivative was dissolved in dry methylene chloride (75 mL), the solution was cooled to −40 °C, and then 3.0 mL (3.39 g, 31.8 mmol) of freshly prepared[10] 2-chlorotetrahydrofuran was added using a syringe. The solution was allowed to stand at 25 °C, and chromatography (TLC) indicated that the reaction was complete in less than 0.5 h. The brown reaction mixture was concentrated in vacuo, and the syrupy residue was dissolved in a hot mixture of chloroform and acetone (7:3, v/v), which was then applied to the top of a dry-packed silica gel column as described above. Elution was carried out with a chloroform–acetone mixture (7:3, v/v), and 40-mL fractions were collected. Fractions 5–15 contained the product, which was isolated in the usual manner and yielded 1.55 g (22.5%) of product: mp 165–166.2 °C after a recrystallization from methyl ethyl ketone; ^{1}H NMR (Me_2SO-d_6) δ 8.13 (s, 1 H, H_5), 7.25 (br s, 2 H, $CONH_2$), 5.98 (t, 1 H, $H_{1'}$, $J_{1',2'}$ = 5 Hz), 3.51 and 3.31 [v br s, 3 H, $N(CH_3)_2$]; UV ($\epsilon \times 10^{-3}$) λ_{max} (pH 1) 314 nm (ϵ 15.1), λ_{min} 352.8 (5.6); λ_{max} (CH_3OH) 308 (14.2), λ_{min} 252.5 (7.8); λ_{max} (pH 11) 309 (13.9), 235.5 (14.1), λ_{min} 254.3 (8.7). Anal. Calcd for $C_{10}H_{16}N_6O_2$: C, 47.57; H, 6.39; N, 33.31. Found: C, 47.62; H, 6.64; N, 33.57.

3-(3,3-Dimethyl-1-triazeno)-1-(tetrahydropyran-2-yl)-pyrazole-4-carboxamide (10b) and 5-(3,3-Dimethyl-1-triazeno)-1-(tetrahydropyran-2-yl)pyrazole-4-carboxamide (11b). Dry 3-(3,3-dimethyl-1-triazeno)pyrazole-4-carboxamide (**3**; 4.0 g, 21.8 mmol) was trimethylsilylated with hexamethyldisilazane as described previously.[9] The solid trimethylsilyl derivative was dissolved in dry methylene chloride (50 mL), and 3.26 mL (3.99 g, 33.1 mmol) of freshly prepared[10] 2-chlorotetrahydropyran was added using a syringe. The reaction was nearly complete in 3 h (TLC); however, the mixture was stored for 48 h at +5 °C in a sealed flask. The pale-yellow solution was concentrated in vacuo to a syrup, which crystallized when ethyl acetate (10 mL) was added. However, this solid (2.1 g; mp 146–185 °C) was a mixture of compounds, as indicated by chromatography (TLC). The solid and supernatant were recombined, dissolved in chloroform (8 mL), and applied to the top of a glass column (2 × 20 cm) dry packed with silica gel (80 g). The column was eluted with a chloroform–acetone mixture (4:1, v/v), and 17-mL fractions were collected. Fractions 15–46 were combined and concentrated to dryness to yield 2.9 g of the faster running isomer (TLC). This solid was recrystallized from hot methyl ethyl ketone (40 mL) to give 1.85 g (31.8%) of **10b** as a white solid, mp 178–179.5 °C. Concentration of the supernatant to 18 mL gave a second crop of crystals (**10b**): yield 770 mg (13.2%); mp 179–180.5 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.14 (s, 1 H, H_5), 7.30 (br s, 2 H, $CONH_2$), 5.34 (dd, 1 H, $H_{1'}$, J = 3.5 and 8.0 Hz), 3.52 and 3.22 [2 s, 6 H, $N(CH_3)_2$]; UV ($\epsilon \times 10^{-3}$) λ_{max} (pH 1) 309 nm (ϵ 13.8), λ_{min} 253.0 (7.0); λ_{max} (CH_3OH) 303.5 (13.4), λ_{min} 252.0 (7.5); λ_{max} (pH 11) 307 (14.6), 236.2 (13.0), λ_{min} 253.1 (8.2). Anal. Calcd for $C_{11}H_{18}N_6O_2$: C, 49.61; H, 6.81; N, 31.56. Found: C, 49.60; H, 6.83; N, 31.73.

The slower moving isomer was obtained from fractions 52–72. Recrystallization from methyl ethyl ketone (20 mL) gave pure **11b** as a white solid, 700 mg (12%), mp 181–182 °C. An additional amount (110 mg, 1.9%) of **11b** was obtained when the supernatant was concentrated to 5 mL: mp 180.5–181.5 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.74 (s, 1 H, H_5), 7.19 (br s, 2 H, $CONH_2$), 5.61 (dd, 1 H, $H_{1'}$, J = 1.8 and 9.1 Hz), 3.60 and 3.27 [2 br s, 6 H, $N(CH_3)_2$]; UV ($\epsilon \times 10^{-3}$) λ_{max} (pH 1) 316 nm (ϵ 15.6), λ_{min} 252.8 (5.8); λ_{max} (CH_3OH) 322.0 (12.7), λ_{min} 250.5 (5.8); λ_{max} (pH 11) 319.5 (5.7), 231.8 (17.0), λ_{min} 258 (6.3). Anal. Calcd for $C_{11}H_{18}N_6O_2$: C, 49.61; H, 6.81; N, 31.56. Found: C, 49.72; H, 6.82; N, 31.81.

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Synthesis and Antitumor Properties of 7-Deoxy-7-[(*cis*- and *trans*-3-aminocyclohexane)thio]carminomycinone

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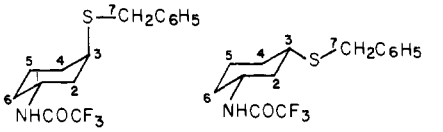
The synthesis of analogues of carminomycin in which the daunosamine group has been replaced by (*cis*- and *trans*-3-aminocyclohexane)thio moieties is described. The new compounds were found to exhibit none of the antitumor or antibiotic activity associated with carminomycin.

The recent disclosure by Tong et al.[1] of the preparation of 7-[(aminoalkyl)thio] derivatives of daunomycinone prompts us to report the synthesis of similar derivatives in the carminomycin series. We sought to replace the daunosaminyl residue of carminomycin (1) with the (3-aminocyclohexane)thio moiety with the expectation that the C_1–S bond would be considerably more stable in vivo than is the glycosidic linkage in 1. We also hoped that the C_7–S bond might prove more resistant to in vivo reduction than the C_7–O bond in 1. In view of the close steric relationship of the analogue to the parent drug, it was expected that a stable complex with DNA could be formed by the analogue.[2]

Chemistry. The desired 3-aminocyclohexanethiols were prepared in the following manner. Base-catalyzed addition of benzyl mercaptan to cyclohexenone (**5**) proceded smoothly to give **6**, which was converted directly to the oxime acetate **7** without further purification. Reduction of **7** with diborane gave the desired *S*-benzyl-3-aminocyclohexanethiol (**8**) as a mixture of cis and trans isomers in which the cis isomer slightly predominated. The overall yield of **8** from **5** was 87%. The isomer ratios were determined by measuring the peak height of the ^{1}H NMR signals for the S-CH_2Ph group for each isomer (at δ 3.69 for the trans isomer **8a** and δ 3.74 for the cis isomer **8b** in $CDCl_3$). Since we anticipated coupling of the thiols to carminomycinone (**2**) as their *N*-trifluoroacetamides, the mixture of amines **8** was converted to the amides **9** via reaction with trifluoroacetic anhydride. While both the amine mixture and the amide mixture were not resolvable by TLC (silica gel using a variety of developing solvents), base-line resolution of the amide isomer mixture was achieved on μ-Porasil high-performance LC columns using methylene chloride–hexane (9:1) as the eluting solvent [k' (isomer 1) = 1.08; k' (isomer 2) = 1.31]. The analytical separation was scaled to a Prep LC/500 scale (5-g loads). The samples were recycled seven times, with the front and back of the main band being shaved on each pass. Excellent material recovery was experienced, and each isomer was obtained enriched to 95% purity. Analysis of the ^{1}H and ^{13}C NMR spectra of the isomers permitted assignment of the trans stereochemistry to the minor isomer 1 and cis stereochemistry to isomer 2.

The ^{1}H NMR spectrum of **9b** exhibited features compatible with a conformationally rigid cyclohexane ring, while the spectrum of **9a** was consistent with an equilibrating set of conformers as evidenced by the methylene envelope between δ 1.3 and 2.0 integrating for 8 protons. In **9b**, the peak width at half-height for the C*H*-S signal was ~18 Hz, which is consistent for an axial proton. The peak widths at half-height for the C*H*-N and C*H*-S signals in the ^{1}H NMR spectrum of **9a** were 18 and 14 Hz respectively, suggesting that the conformer of **9a** in which C–S bond is axial is the major contributor to the time-averaged spectrum of **9a**. These assignments were confirmed by an analysis of the ^{13}C NMR spectra of **9a** and **9b**. The ^{13}C NMR assignments were made on the basis of model compounds from the literature and theoretical considerations.[3] The observed line positions, together with their assignments, are listed in Table I. It has been observed that sterically crowded atoms in a cyclohexane ring, such as the 1, 3, and 5 carbons in cyclohexanes carrying an axial substituent, are found at higher field than similar carbon atoms not subject to steric compression effects. Thus, **9a** having carbon-1, -3, and -5 at highest field was assigned to the trans isomer in agreement with the ^{1}H NMR assignment. While C_1 and C_5 exhibit normal compression shifts of ~4.4 ppm on going from **9b** to **9a**, C_3 shows only a minimal compression shift of 1.4 ppm. This is further support of the suggestion from the ^{1}H NMR spectrum that **9a**-A is the major conformer of **9a** in solution.

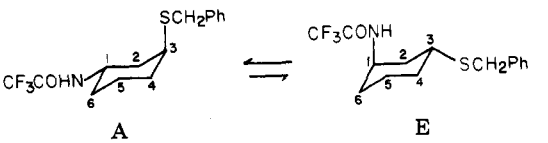
Table I. ^{13}C NMR Listings for 9a and **9b**[a]



9a 9b

carbon	9a	9b
1	45.9	50.4
2	40.8	43.5
3	31.1	32.5
4	35.3	35.9
5	20.4	24.7
6	35.3	34.5
7	40.2	41.1

[a] In parts per million downfield from Me_4Si. Recorded in $CDCl_3$.



A E

The ratio of cis to trans isomers of **8** produced on diborane reduction of the oxime deserves some comment. While a variety of methods for the reduction of oximes to

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