has a pyramidal equilibrium geometry. We predict a bond angle of 111.6° and an inversion barrier of 2.3 kcal mol^{-1}. These results are consistent with recent neutron diffraction findings in the solid state (yielding HOH bond angles of 110.4° for *p*-$CH_3C_6H_4SO_3^-H_3O^+$ and 112.7° for $F_3CSO_3^-H_3O^+$),[16] and Symons' interpretation of the ^{17}O NMR spectrum of H_3O^+ (yielding a bond angle of 111.3°).[2]

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Triplet GpCpA Forms a Stable RNA Duplex

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Perfect RNA duplexes containing three Watson–Crick base pairs are unstable under physiological conditions.[1] Triribonucleotides, however, can form stable duplexes with single stranded helical regions, such as in tRNA loops where the bases already stacked in the helix account for this enhanced stability.[2] An alternate means of increasing base stacking and thus strengthening overall duplexes is the presence of dangling bases.[3] We report the first triribonucleotide to form a simple stable duplex, GpCpA:GpCpA, which contains two G·C Watson–Crick base pairs and two 3′-dangling adenosines. This duplex is similar in stability to the corresponding self-complementary tetramer duplex, formed from UpGpCpA, which contains four Watson–Crick pairs, and must derive its stability over the dinucleotide duplex GpC:GpC by virtue of its 3′-dangling adenosine residues whose contributions to duplex stability approximate those of A·U pairs.

Oligoribonucleotides, GpC, GpCpA, GpCpApA, and ApGpC, were synthesized by using a phosphotriester method.[4] Variable-temperature ^1H nuclear magnetic resonance spectroscopy was used to monitor duplex stability. The chemical shift vs. temperature changes for the aromatic and ribose H-1′ protons of GpCpA are shown in Figure 1 and listed in Table I. The averaged T_m for the sigmoidal plots of these protons is 33 °C at 7.3 mM. The plots displayed in Figure 1 are only consistent with a GpCpA duplex containing two Watson–Crick base pairs. The chemical shift of CH-5 changes by 0.615 ppm to higher field over the temperature range 70–10 °C, and this upfield movement is characteristic of a CH-5 on a cytidine which is involved in a normal G·C Watson–Crick base pair as is shown by the 0.559-ppm upfield shift for the CH-5 in the UGCA duplex (see Table II). Protons, AH-8 and AH-2, of the dangling adenosines exhibit pronounced upfield chemical shift changes during GpCpA duplex formation. In addition, the $J_{1',2'}$ coupling constants for the ribose H-1′ protons of the guanosine and cytidine residues collapse to <0.5 Hz below 30 °C, while the $J_{1',2'}$ values for adenosine decrease but do not become <0.5 Hz until close to 0 °C. This is indicative of strong GC stacking, while the 3′-adenosine unit still retains some flexibility in the duplex.[5]

Although the trimer, GpCpA, contains a purine–pyrimidine–purine sequence, these results provide an interesting contrast to those obtained from studies of similar type of base sequence which preferred internal bulge base conformations[6] at lower temperatures.

The spectacular stability of the GpCpA duplex containing a 3′-dangling adenosine is even more dramatic when compared to the trinucleotide ApGpC containing a 5′-dangling adenosine.

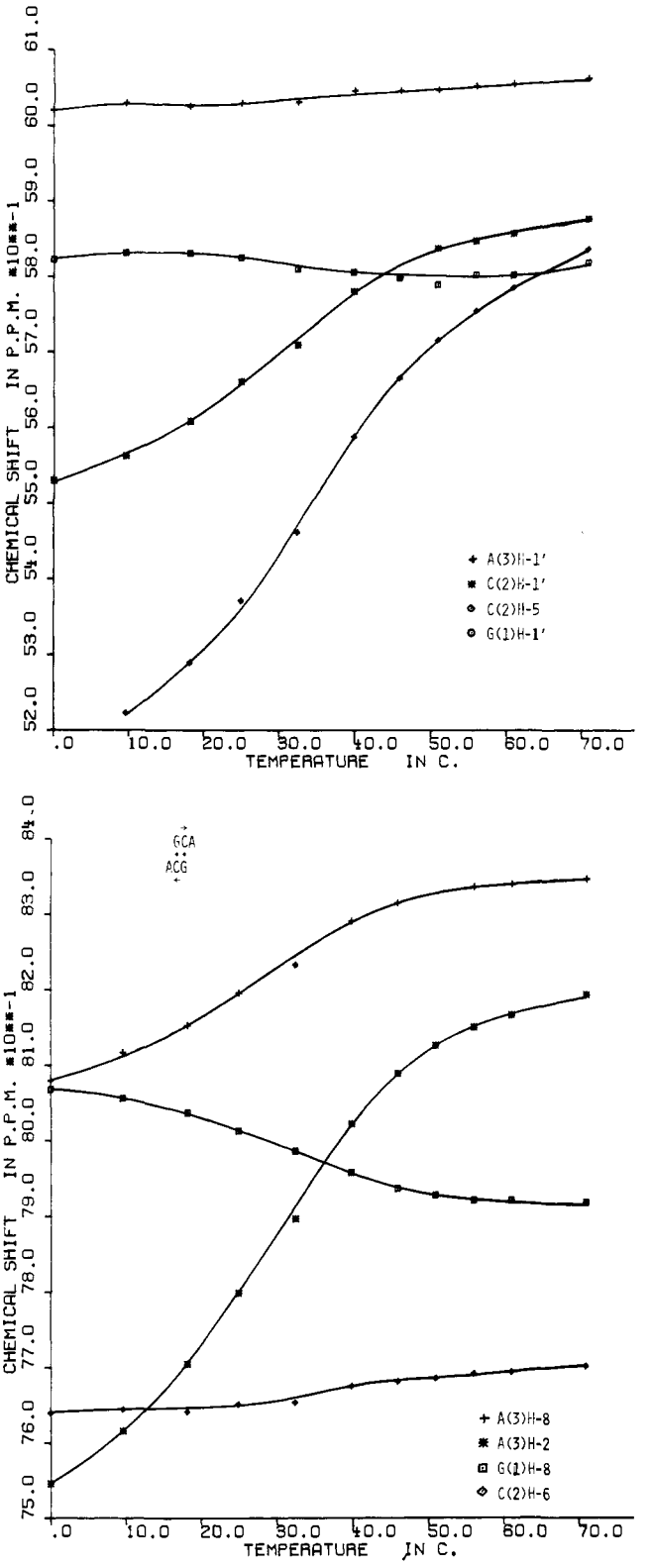

Figure 1. Chemical shift vs. temperature plots for GpCpA at 7.3 mM. Sample was dissolved in 100% D_2O containing 0.01 M sodium phosphate buffer (pD 7.0) and 1.0 M sodium chloride.

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Table I. NMR Chemical Shift Assignments for GCA (7.3 mM) over the Temperature Range 70–0 °C

	temperature, °C								
resonance	70.6	59.6	47.9	38.5	27.1	20.0	12.0	0.6	T_m
A(3)H-8	8.348	8.340	8.310	8.264	8.191	8.156	8.120	8.085	30.5
A(3)H-2	8.191	8.153	8.069	7.955	7.790	7.703	7.624	7.564	32
G(1)H-8	7.920	7.925	7.941	7.969	8.009	8.031	8.045	8.053	32
C(2)H-6	7.709	7.699	7.685	7.669	7.647	7.642	7.641	7.634	NSB[a]
A(3)H-1'	6.061	6.057	6.046	6.036	6.027	6.022	6.017	6.010	NSB
C(2)H-1'	5.883	5.850	5.808	5.744	5.652	5.600	5.554	5.516	32
G(1)H-1'	5.812	5.807	5.800	5.801	5.811	5.817	5.812	5.798	NSB
C(2)H-5	5.837	5.774	5.659	5.527	5.362	5.287	5.216		39
									av T_m = 33

[a] NSB = no sigmoidal behavior.

Table II. NMR Chemical Shift Assignments for UGCA (8.2 mM) over the Temperature Range 70–0 °C

	temperature, °C										
resonance	70.6	62.0	53.3	43.4	38.5	33.4	27.9	19.8	12.1	2.5	T_m
AH-8	8.362	8.359	8.349	8.320	8.274	8.232	8.167	8.092	8.014	7.977	30.5
AH-2	8.202	8.183	8.147	8.066	7.978	7.887	7.760	7.616	7.463	7.369	30.5
GH-8	7.975	7.975	7.978	7.984	7.978	7.978	7.958	7.919	7.864		40.5
UH-6	7.716	7.727	7.747	7.793	7.819	7.845	7.874	7.874	7.825	7.781	NSB
CH-6	7.711	7.700	7.692	7.675	7.664	7.661	7.642	7.646	7.638		NSB
AH-1'	6.068	6.063	6.062	6.057	6.049	6.045	6.039	6.027	6.027	6.021	32.0
CH-1'	5.875	5.865	5.845	5.808	5.777	5.812	5.812	5.819	5.806		NSB
CH-5	5.853	5.806	5.749	5.640	5.565	5.488	5.407	5.335	5.259	5.221	38.0
UH-5	5.811	5.798	5.790	5.793	5.791	5.798	5.798	5.782	5.764	5.749	NSB
UH-1'	5.811	5.803	5.811	5.769	5.733	5.682	5.627	5.511	5.477	5.467	30.5
GH-1'	5.794	5.812	5.811	5.769	5.770	5.739	5.627	5.526	5.646	5.524	32.0
											av T_m = 33

Table III. NMR Chemical Shift Assignments for AGC (16 mM) over the Temperature Range 70–0 °C

	temperature, °C							
resonance	72.1	60.6	50.0	37.7	31.7	20.6	9.6	0.4
AH-8	8.238	8.235	8.235	8.227	8.224	8.213	8.191	8.191
AH-2	8.170	8.143	8.116	8.072	8.050	8.004	7.945	7.909
GH-8	7.934	7.909	7.882	7.842	7.820	7.768	7.687	7.627
CH-6	7.748	7.737	7.725	7.707	7.698	7.677	7.643	7.627
AH-1'	5.969	5.960	5.953	5.935	5.926	5.899	5.846	5.821
CH-5	5.872	5.834	5.788	5.724	5.698	5.639	5.533	5.493
CH-1'	5.889	5.866	5.854	5.834	5.826	5.802	5.767	5.740
GH-1'	5.809	5.778	5.754	5.702	5.678	5.630	5.566	5.526

Table IV. NMR Chemical Shift Assignments for GCAA (7.3 mM) over the Temperature Range 70–0 °C

	temperature, °C										
resonance	70.8	60.8	51.0	45.9	40.5	35.7	30.2	25.1	19.6	9.4	T_m
A(3)H-8	8.313	8.306	8.278	8.257	8.224	8.166	8.098	8.080	8.074	8.047	36
A(4)H-8	8.313	8.306	8.278	8.257	8.224	8.190	8.138	8.120	8.117	8.047	38.5
A(4)H-2	8.181	8.166	8.150	8.138	8.126	8.114	8.098	8.080	8.074	8.047	35
A(3)H-2	8.101	8.065	8.001	7.943	7.857	7.760	7.632	7.516	7.418	7.256	31
GH-8	7.909	7.912	7.921	7.928	7.949	7.967	7.989	8.013	8.045	8.047	31
CH-6	7.717	7.713	7.707	7.699	7.688	7.676	7.646	7.640	7.635	7.625	35
A(4)H-1'	6.038	6.024	6.001	5.979	5.957	5.932	5.884	5.874	5.880	5.874	33.5
A(3)H-1'	5.977	5.957	5.940	5.930	5.917	5.905	5.884	5.832	5.829	5.789	NSB
CH-5	5.849	5.808	5.739	5.685	5.612	5.530	5.437	5.357	5.283		36
CH-1'	5.867	5.849	5.817	5.794	5.757	5.713	5.657	5.609	5.572	5.517	33
GH-1'	5.809	5.793	5.806	5.794	7.795	5.803	5.803	5.808			
											av T_m = 34

Chemical shift vs. temperature changes for the aromatic and ribose H-1' protons of ApGpC at 16.0 mM are shown in Table III. The small chemical shift changes observed are typical for all reported trinucleotides[6,7] and implies that for the molar concentration range (1–10 mM) the averaged T_m value would be <0 °C. The behavior of the CH-5 proton of ApGpC is also indicative of there being no significant interaction of cytidine and guanosine in the normal Watson–Crick manner at temperatures down to 0 °C. Observations that 3'-dangling residues contribute more to duplex stability than the corresponding 5'-dangling residues have been previously reported.[3,8] The greater helical overlap of a 3'-base residue generates increased aromatic ring–current interaction within a strand, enhancing base stacking which in turn strengthens duplex formation. Our own unpublished studies indicate that within single strands, chemical shift parameters for 3'-adjacent bases are greater than those for corresponding 5' neighbors. Larger shift parameters[9] are used for flanking 3' neighbors when assigning ring

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protons in A·U and G·C hydrogen-bonded systems.

Comparison of GpCpA with UpGpCpA is significant. The chemical shift vs. temperature data for the aromatic and ribose H-1′ protons of UpGpCpA at 8.2 mM are contained in Table II, and its average T_m was 33 °C. Remarkably the GpCpA duplex which contains only two Watson–Crick base pairs and two dangling adenosine residues is equal in stability to the UpGpCpA duplex which contains four Watson–Crick base pairs. We consider that a combination of factors, base-stacking, hydrophobic interactions, solvation and entropic effects, as well as Watson–Crick hydrogen bonding, contribute to duplex stability.

Stability of the GpCpApA duplex was also studied and its T_m found to be 34 °C at 7.3 mM (Table IV). Behavior was similar to that for GpCpA, and its was noteworthy that the effects of 3′-terminal dangling adenosines were cooperative. However, the residue immediately adjacent to the base-paired region appears to make a major contribution to duplex stability.

Acknowledgment. We thank Ian Wigle for developing the computer analysis in the determination of T_m values. This research was supported by NSERC of Canada.

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Synthesis of a Thiacyclopentyne

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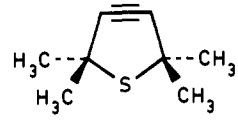
As a step in a program of generating and studying sulfur-containing reactive intermediates,[1,2] we undertook the synthesis of **1**. This was a reasonable objective since good evidence for



1

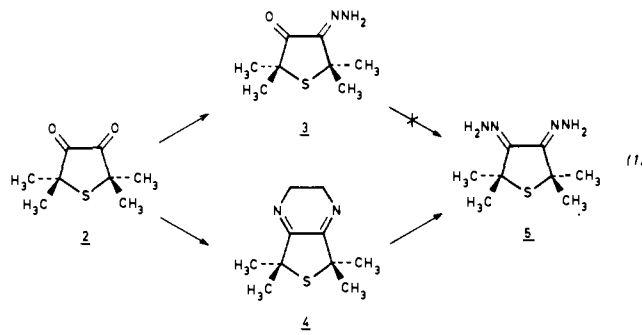
the existence of cyclopentyne as a short-lived intermediate is available.[3-5] Strained cycloalkynes and arenes remain matters of fundamental, theoretical, and synthetic interest to organic chemistry.[6] It seems likely that the ring strain in **1** will be less than in cyclopentyne owing to the longer carbon–sulfur bonds. The methyl groups should sterically shield the reactive triple bond much as in stable 3,3,6,6-tetramethyl-1-thiacycloheptyne[7] or 3,3,7,7-tetramethylcycloheptyne.[8] We also thought it possible that the carbon–sulfur–carbon σ bond segment could stabilize the heavily distorted in-plane π system wherein much of the strain is located.[9] On the negative side, the possibility is present that **1**, if generated, would immediately eliminate the sulfur bridge.

Table I. Yields of Products Obtained from Oxidation of Dihydrazone under Various Conditions

	yield, %[a]				
experiment	7	8	9	10	11
A[b]	28.4	15.3	9.4		
B[c]	7.4	7.8	4.1	6.9	
C[d]	10.8	6.3	3.6		12.6
D[e]			48.5		4.1
E[f]			54		

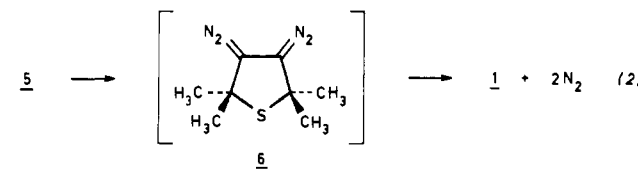
[a] Yields determined by ^{1}H NMR using $CH_3SO_2CH_3$ as internal standard; the balance of the materials consisted of intractable tar. [b] Oxidation with $Pb(O_2CCH_3)_4$ in CH_2Cl_2 under N_2 at 0 °C. [c] Oxidation with $Pb(O_2CCH_3)_4$ at 0 °C under N_2 in pure redistilled $C_6H_5N_3$. [d] Oxidation with $Pb(O_2CCH_3)_4$ at 20 °C under N_2 in pure redistilled 2,5-dimethylfuran. [e] Oxidation with MnO_2 at 20 °C under N_2 in pure redistilled 2,5-dimethylfuran. [f] Oxidation with MnO_2-$2H_2O$ in CH_2Cl_2 under N_2 at 20 °C.

The route followed to **1** is classical. Diketone **2**, the synthesis of which has been reported,[10] was converted to the dihydrazone **5** (eq 1). Direct treatment of **2** with H_2NNH_2,H_2O, $H_2NNH_3^+,HSO_4^-$ gave monohydrazone **3**, which was not stable to the required forcing conditions[11] and decomposed rather than providing **5**. An indirect route adapted from an earlier work of van Alpen[12] involving formation of dihydropyrazine **4**[13] and subsequent conversion (H_2NNH_2,H_2O, $H_2NNH_3^+,HSO_4^-$, ethylene glycol, 120 °C, 4 h) was successful and gave **5** in 65% overall yield.



(1)

The dihydrazone **5** was subjected to oxidation. Bis(diazo) compound **6** is assumed to be formed and this should be a precursor of **1** (eq 2).[2-5] Depending on the reaction conditions and additives used, the products **7**–**11** were obtained. All these



(2)

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