Two of eight antibodies were found to catalyze the reduction of α-keto amide **1** to the α-hydroxy amide **2**. One antibody, A5, was examined in greater detail.

The antibody-catalyzed, $NaBH_3CN$-dependent reduction displayed a pH optimum at acidic pH; consequently, all kinetic parameters were measured in the presence of 13 μM antibody at 22 °C in 50 mM NaCl, 50 mM MES buffer, pH 5.0.[14] The antibody-catalyzed reaction could be described by the following kinetic scheme:

$$\mathrm{Ig} + \mathrm{S} \overset{K_M}{\rightleftharpoons} \mathrm{Ig{\cdot}S} \xrightarrow[\mathrm{NaBH_3CN}]{k_{cat,app}} \mathrm{Ig} + \mathrm{P}$$

where S is α-keto amide substrate **1**, Ig is antibody A5, K_M is the Michealis constant for **1**, and $k_{cat,app}$ is the k_{cat} (catalytic constant) observed at a particular fixed concentration of $NaBH_3CN$. A Lineweaver-Burk analysis of the steady-state kinetic data at 1 mM $NaBH_3CN$ afforded a k_{cat} of 0.104 min^{-1} and a K_M(**1**) of 1.24 mM. The pseudo-first-order rate constant for the uncatalyzed reaction (k_{uncat}) at 1 mM $NaBH_3CN$ in the same buffer was found to be 3.6×10^{-4} min^{-1}. The antibody-catalyzed reaction was inhibited by 4-nitrophenyl methyl phosphate: the K_d was determined from fluorescence quenching experiments to be 0.61 μM. Greater than 25 turnovers were measured with no apparent change in V_{max}, suggesting that $NaBH_3CN$ does not inactivate the antibody at a significant rate.

The diastereomeric excess of the reaction was determined by extraction of product into methylene chloride followed by acetylation with acetic anhydride/pyridine/DMAP and subsequent analysis using capillary gas chromatography.[16] Product stereochemistry was assigned by comparison to authentic products.[17] Controls demonstrated that the diastereomeric composition of products **2R** and **2S** was stable to the workup and assay conditions. The uncatalyzed reaction afforded α-hydroxy amide **2R** with a diastereomeric excess of 56%. In contrast, the antibody-catalyzed reaction afforded the product **2S** with a diastereomeric excess greater than 99% (*opposite* the stereospecificity of the uncatalyzed reaction), indicating that the antibody combining site discriminates the enantiomeric transition states for carbonyl reduction with high selectivity. Further screening is likely to provide antibodies with a broad array of selectivities including specificity for the product **2R**.

Future experiments will explore antibody-catalyzed, metal hydride dependent carbonyl and imine reductions; regiospecificity; substructure selectivity; and improvements in hapten design (including the use of sulfoxides, phosphinates, and phosphonate diesters). In addition, the use of other powerful synthetic reagents in conjunction with antibody catalysis is being explored.

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(13) Westerberg, D. A.; Carney, P. L.; Rogers, P. E.; Kline, S. J.; Johnson, D. K. *J. Med. Chem.* **1989**, *32*, 236.

(14) Stock solutions of **1** (5 mM) and 10 μL of $NaBH_3CN$ (50 mM) in MeOH were added to 0.5 mL total volume of reaction buffer containing 2 mg/mL antibody and 10% (v/v) MeOH. Initial reaction rates were determined by measuring the amounts of **1** and **2** (both diastereomers) relative to a *p*-cresol internal standard using analytical reverse-phase HPLC (Rainin Dynamax Microsorb C_{18}, 40-80% of 0.06% TFA/CH_3CN in 0.1% aqueous TFA).

(15) The dissociation constant was determined from a Scatchard analysis of fluorescence titrations (λ_{ex} = 280 nm, λ_{em} = 346 nm) using 0.14 mg/mL A5 in assay buffer.

(16) Stock solutions of 20 μL of **1** (5 mM) and 10 μL of $NaBH_3CN$ (50 mM) in MeOH were added to 0.5 mL total volume of 10 mg/mL antibody containing 10% (v/v) MeOH and shaken for 4 h. After extraction of the reaction mixture with CH_2Cl_2 and removal of solvent, the residue was dissolved in CH_3CN and acetylated with acetic anhydride, pyridine, and DMAP. The aceylated products were purified by reverse-phase HPLC (Rainin Dynamax Microsorb C_{18}, 30-80% of 0.06% TFA/CH_3CN in 0.1% aqueous TFA) prior to analysis by capillary GC (HP-1, cross-linked methyl silicone gum, 25 m × 0.2 mm × 0.33 μm film thickness, FID detector), using (*S,S*)-*N*-(α-methylbenzyl)-*O*-acetyl-3-phenyllactamide as an internal standard. The diastereomeric excess of the antibody-catalyzed reaction was corrected for background reaction.

(17) (*S,S*)-*N*-(α-Methylbenzyl)-*O*-acetyl-3-(4-nitrophenyl)lactamide (**2S**) was prepared from (*S*)-(−)-3-(4-nitrophenyl)lactic acid[18] by condensation of the NHS ester with (*S*)-(−)-α-methylbenzylamine followed by acetylation and chromatography on silica gel (CH_2Cl_2/EtOAc). Sodium borohydride reduction of α-keto amide **1** afforded product **2R** (as a mixture of **2R** and **2S**).

(18) Koga, K.; Wu, C. C.; Yamada, S. *Tetrahedron Lett.* **1971**, 2287.

Sugar Conformations in Intramolecular DNA Triplexes Determined by Coupling Constants Obtained by Automated Simulation of P.COSY Cross Peaks

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Evidence for RNA triple helices formed from two pyrimidine strands and one purine strand was first reported in 1957.[1] Subsequently evidence for formation of other RNA and DNA triple helices from polynucleotides was reported by several laboratories.[2] The generally accepted base pairing in these structures was a Watson-Crick duplex formed from one purine and one pyrimidine strand, with the second pyrimidine strand Hoogsteen base paired to the purine strand. Based on fiber diffraction studies of poly(U)·poly(A)·poly(U)[3] and poly(dT)·poly(dA)·poly(dT),[4] Arnott and co-workers concluded that the DNA triplex formed a structure similar to the RNA triplex; that is, an A′ helix with 12 base triplets per turn, an axial rise per residue of 3.26 Å, base tilts of 7-9°, and C3′-endo sugar puckers in all three strands.[4] This model for the structure of DNA triplexes has been widely accepted in the literature.[5] Although the rise per residue and the helical twist can be accurately determined from fiber diffraction data, it should be noted that the sugar conformation cannot be obtained from fiber diffraction due to the low resolution of the data, and the C3′-endo sugar pucker in the triplex model was based on an assumption.[4] In addition, the rise per residue and base tilts are closer to those of B DNA than to those of A′ DNA.[6] In our recent two-dimensional NMR studies on DNA triplexes formed from $d(TC)_4$ and $d(GA)_4$, we confirmed the proposed base-pairing schemes but presented evidence based on NOE intensities that the purine strand did not have N-type (near C3′-endo) sugar puckers.[7] A more reliable estimation of the sugar conformations can only be obtained from analysis of the fine structure of COSY[8] cross peaks.[9] Here we present an analysis of the cross-peak patterns and coupling constants from the phase-sensitive COSY spectrum of a 31-base intramolecular DNA triplex (HD31). Accurate coupling constants were obtained using our new program CHEOPS (*c*oupling constants from *h*igh-resolution

(1) Felsenfeld, G.; Davies, D. R.; Rich, A. *J. Am. Chem. Soc.* **1957**, *79*, 2023-2024.

(2) Reviewed in the following: (a) Felsenfeld, G.; Miles, H. T. *Annu. Rev. Biochem.* **1967**, *36*, 407-448. (b) Michelson, A. M.; Massoulié, J.; Guschlbauer, W. *Prog. Nucleic Acid Res. Mol. Biol.* **1967**, *6*, 83-141. (c) Wells, R. D.; Collier, D. A.; Hanvey, J. C.; Shimizu, M.; Wohlrab, F. *FASEB J.* **1988**, *2*, 2939-2949.

(3) Arnott, S.; Bond, P. J. *Nature, New Biol.* **1973**, *244*, 99-101.

(4) Arnott, S.; Selsing, E. *J. Mol. Biol.* **1974**, *88*, 509-521.

(5) (a) Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645-650. (b) Lyamichev, V. I.; Mirkin, S. M.; Frank-Kamenetskii, M. D.; Cantor, C. R. *Nucleic Acids Res.* **1988**, *16*, 2165-2178. (c) François, J.-C.; Saison-Behmoaras, T.; Hélène, C. *Nucleic Acids Res.* **1988**, *16*, 11431-11440.

(6) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

(7) (a) Rajagopal, P.; Feigon, J. *Biochemistry* **1989**, *28*, 7859-7870. (b) Rajagopal, P.; Feigon, J. *Nature* **1989**, *339*, 637-640.

(8) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229-2246.

(9) Widmer, H.; Wüthrich, K. *J. Magn. Reson.* **1987**, *74*, 316-336.

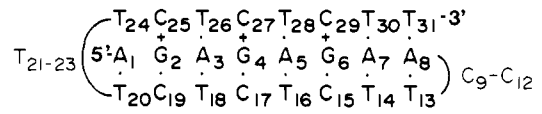
0002-7863/92/1514-781$03.00/0

Table I. Coupling Constants for the Sugar Protons on the Nucleotides Involved in Base Triplets in HD31 Derived from the H1′–H2′ and H1′–H2″ Cross Peaks Using CHEOPS

	$J_{1'2'}$	$J_{1'2''}$	$J_{2'2''}$	corr[a]	% S[b]	P_S[c]	P_N[c]
A1	–	–	–	–	–		
G2	–	–	–	–	–		
A3	10.1	4.9	−13.1	0.941	94	146	
G4	10.5	2.5	−11.6	0.966	100[d]	152	
A5	10.0	4.1	−13.1	0.946	86	146	
G6	10.6	3.5	−14.0	0.964	100[d]	137	
A7	7.4	6.2	−14.8	0.972	83	121	
A8	8.3	4.2	−14.0	0.928	74	138	−28
T13	8.5	5.8	−14.1	0.939	71	154	5
T14	8.4	5.5	−15.2	0.952	79	124	
C15	7.1	5.0	−14.7	0.934	71	131	−13
T16	9.4	6.1	−13.7	0.927	96	179	
C17	5.5	7.7	−16.6	0.941	52[d]	150	42
T18	7.0	7.2	−15.5	0.940	73[d]	136	17
C19	6.5	5.4	−15.4	0.945	59	146	−48
T20	7.9	4.6	−13.9	0.920	76	146	
T24[e]	9.0	5.3	−13.4	0.944	84	149	
C25	5.0	6.1	−15.9	0.939	69[d]	127	−6
T26	8.3	6.0	−15.2	0.941	74	148	14
C27	4.8	6.1	−15.0	0.940	41	128	−23
T28	9.4	4.6	−14.2	0.946	87	145	
C29	4.9	6.4	−15.6	0.944	47	182	−29
T30	8.0	5.7	−15.9	0.932	80	139	3
T31	7.3	7.1	−15.8	0.934	56[d]	149	71

[a] Correlation coefficient. [b] Percent S-type sugar conformation calculated as described.[18] [c] Pseudorotation angle calculated for S-type and N-type sugars.[18] [d] RMS difference between CHEOPS coupling constants and PSEUROT coupling constants >1, due either to unusually low $J_{1'2''}$ couplings (caused perhaps by abnormal relaxation effects) or to the lesser accuracy in the determination of $J_{2'3'}$ and $J_{2''3'}$ couplings. [e] T24 is not base paired.

Scheme I



NMR *e*xperiments by *o*ptimized *p*arameter *s*imulation), which simulates selected regions of the COSY spectrum by automatically iteratively varying coupling constants, line widths, and chemical shifts to maximize the fit between measured and simulated spectra. The results show that although both N-type (near C3′-endo) and S-type (near C2′-endo) sugar puckers[6] are present, the majority of sugars in this molecule are predominantly S-type.

The 31-base DNA oligonucleotide (HD31) folds to form an intramolecular triplex as shown in Scheme I (plus sign indicates protonated C at N_3), similar to those we have reported elsewhere.[10] A visual examination of the H1′–H2′,H2″ region of the P.COSY[11] spectrum of this molecule immediately revealed that the majority of the sugars do not show the cross peak patterns expected for N-type sugars. For N-type sugars, large couplings are expected for H1′–H2″ and small couplings for H1′–H2′, while the reverse should be true for S-type sugars.[12] The quantitative determination of the coupling constants was performed by simulation of the H1′–H2′,H2″ cross peaks from the P.COSY spectrum of the triplex. To avoid the time-consuming and possibly biased manual comparison of experimental and simulated cross-peak patterns, a fully automated procedure similar to but more practical than the method of Mādi and Ernst[13] was developed. This program (CHEOPS) performs spectral simulations using the SPHINX software[14] and employs Powell's algorithm[15] to maximize the correlation coefficient between calculated and measured spectral intensities. An important advantage of the program is that reasonable coupling constants are obtained even for overlapping cross peaks by simultaneously simulating several spin systems. In addition, the correlation coefficient gives a quantitative measure of agreement between simulated and experimental data that is independent of scaling.[15] The program required less than 1 day of CPU time on a Silicon Graphics IRIS 4D/25 to simulate the H1′–H2′,H2″ spin systems of HD31.[16] Complete details of this method will be presented elsewhere (Schultze and Feigon, manuscript in preparation).

The deoxyribose coupling constants obtained by simulation of the H1′–H2′,H2″ region of the P.COSY spectrum of HD31 using CHEOPS are presented in Table I for the nucleotides in the base triplets. A rough estimate of the % S sugar pucker is also given, calculated using the program PSEUROT.[17,18] This analysis indicates that all of the purines and thymidines (except T_{31}) are predominantly S-type. Only some cytidines, especially those in the third strand, have a large proportion of the N-type conformation. These results, along with the more qualitative results obtained from MINSY[19] spectra on other DNA triplexes,[7,20] indicate that the DNA does not need to adopt all A-DNA-type sugar puckers to accommodate the third strand. A more detailed analysis of the sugar conformations in this intramolecular triplex will be presented elsewhere.[21]

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(10) (a) Sklenář, V.; Feigon, J. *Nature* **1990**, *345*, 836–838. (b) Macaya, R.; Gilbert, D. E.; Malek, S.; Sinsheimer, J. S.; Feigon, J. *Science* **1991**, *254*, 270–274.
(11) Marion, D.; Bax, A. *J. Magn. Reson.* **1988**, *80*, 528–533.
(12) Schmitz, U.; Zon, G.; James, T. L. *Biochemistry* **1990**, *29*, 2357–2368.
(13) Mādi, Z. L.; Ernst, R. R. *J. Magn. Reson.* **1988**, *79*, 513–527.
(14) Widmer, H.; Wüthrich, K. *J. Magn. Reson.* **1986**, *70*, 270–279.
(15) Press, W. H.; Flannery, B. P.; Teukolsky, S. A.; Vetterling, W. T. *Numerical Recipes*; Cambridge University Press: Cambridge, 1989.
(16) Cross peaks from G_2 and T_{21} were both too weak to be simulated. Accurate coupling constants could not be obtained for A_1 since its H1′–H2′ and H1′–H2″ cross peaks overlap.
(17) (a) van Wijk, J.; Huckriede, B. D.; Ippel, J. H.; Altona, C. *Methods Enzymol.*, in press. (b) de Leeuw, F. A. A. M.; Altona, C. *J. Comput. Chem.* **1983**, *4*, 428–437.
(18) The deoxyribose conformations were analyzed assuming a fast, two-state equilibrium between S- and N-type conformations. With the program PSEUROT a least-squares fit was optimized between the coupling constants obtained from the COSY simulation (including $J_{2'3'}$ and $J_{2''3'}$) and the theoretical values as a function of the pseudorotation phase angles P_N and P_S and the molar fraction of the two states. Both ring pucker amplitudes were kept constant at 36°. The P_N value was considered not sufficiently determined in sugar residues in which its molar fraction was found to be less than 25%.
(19) Massefski, W., Jr.; Redfield, A. G. *J. Magn. Reson.* **1988**, *78*, 150–155.
(20) Mooren, M. M. W.; Pulleyblank, D. E.; Wijmenga, S. S.; Blommers, M. J. J.; Hilbers, C. W. *Nucleic Acids Res.* **1990**, *18*, 6523–6529.
(21) Macaya, R. F.; Wang, E.; Schultze, P.; Sklenář, V.; Feigon, J. *J. Mol. Biol.*, in press.

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Supplementary Material Available: Region of P.COSY spectrum used for simulations and table including $J_{2'3'}$ and $J_{2''3'}$ from simulation (5 pages). Ordering information is given on any current masthead page.

Functionalization of Saturated Hydrocarbons: Selective Insertion Reactions of Dihalocarbenes into Carbon-Hydrogen Bonds Adjacent to Cyclopropane Rings†

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Attempts to selectively functionalize saturated hydrocarbons[1] have been called "the search for the chemist's Holy Grail".[2] The fact that dihalocarbenes can insert into C-H bonds was recognized 30 years ago.[3] Since then, a number of different C-H insertions of dihalocarbenes have been discovered.[4,5] Moderate yields for insertion of dihalocarbenes into *tertiary* C-H bonds of saturated hydrocarbons have been obtained, however, only from ball-shaped molecules, such as adamantane[5h,o] and dodecahedrane.[5v] In contrast, insertions of dihalocarbenes into *secondary* and *tertiary* C-H bonds of other saturated hydrocarbons ordinarily afford only very low yields.[5j,l,p]

Table I. Insertion Reactions of $:CX_2$ into Carbon-Hydrogen Bonds of Small Ring Compounds

small ring compd	X	products (ratios, %)[a]	yield (%)[b]
1	Cl	**2a** (81), **2b** (19)	83
	Br[c]	**3a** (80), **3b** (20)	27
4	Cl	**5a** (26), **5b** (74)	40
	Br[c]	**6a** (20), **6b** (80)	18
7	Cl	**8a** (83), **8b** (17)	57
9	Cl	three isomers[d]	2
10	Cl	no reaction	0
11	Cl	**12**	90

[a] Relative ratios measured by ^1H NMR spectroscopy. [b] Total yields of two isomers. [c] Stereochemical assignment based on similarity of NMR spectra with the corresponding chloro compounds. [d] Ratios = 5:1:1 based on ^1H NMR of signals of $CHCl_2$ groups.

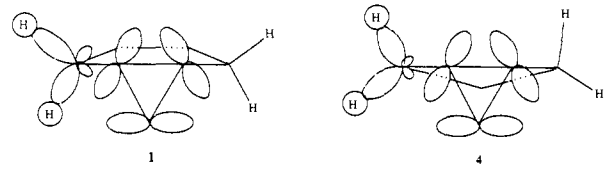
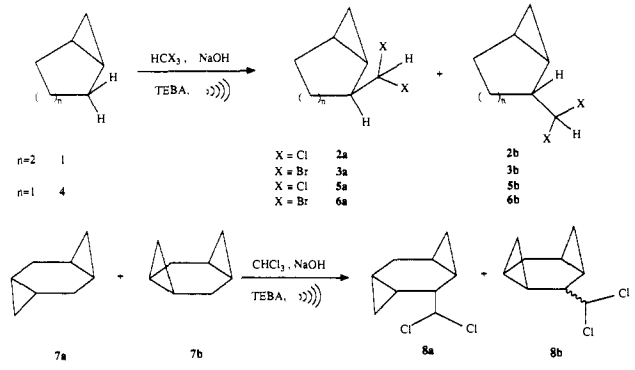


Figure 1.

Scheme I



Although reactions of dihalocarbenes with strained compounds containing three-membered rings have been studied for about 25 years,[6,7] C-H insertion reactions of dihalocarbenes with hydrocarbons containing three- or four-membered rings have not been reported. We sought evidence to support the premise that C-H bonds α to a three-membered ring could be inserted because of the possibility of an interaction of the Walsh orbitals of the cyclopropane ring with suitable C-H bond orbitals.

The reactions of dihalocarbenes with olefins in solid-liquid two-phase systems under ultrasonication usually afford high yields of double-bond addition products.[8,9] When a mixture of bicyclo[4.1.0]heptane (**1**),[10] chloroform, powdered sodium hydroxide, and 0.5% of TEBA (triethylbenzylammonium chloride) was ultrasonicated in the water bath of an ultrasonic cleaner (35 kHz, 120 W) for 3 h, 2-(dichloromethyl)bicyclo[4.1.0]heptanes **2a** and **2b** were obtained in a yield of 83% (ratio = 4.3:1; see Table I). As determined by NOE experiments,[11] the dichloromethyl group

† Carbene Rearrangements. Part 38. Part 37: Xu, L.; Miebach, T.; Smith, W. B.; Brinker, U. H. *Tetrahedron Lett.* **1991**, *32*, 4461-4.

‡ State University of New York at Binghamton.

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(1) Hill, C. L., Ed. *Activation and Functionalization of Alkanes*; Wiley: New York, 1989.

(2) Barton, D. H. R. *Aldrichimica Acta* **1990**, *23*, 3-10.

(3) Parham, W. E.; Koncos, R. *J. Am. Chem. Soc.* **1961**, *83*, 4034-8.

(4) Reviews: (a) Kirmse, W. *Carbene Chemistry*; Academic Press: New York, 1971; pp 209-35. (b) Gaspar, P. P.; Hammond, G. S. In *Carbenes*; Moss, R. A., Jones, M., Jr., Eds.; Wiley: New York, 1975; Vol. 2, pp 310-9. (c) Jones, W. M.; Brinker, U. H. In *Pericyclic Reactions*; Marchand, A. P., Lehr, R. E., Eds.; Academic Press: New York, 1977; pp 118-20. (d) Dehmlow, E. V. In *Houben-Weyl, Methoden der Organischen Chemie*; Regitz, M., Ed.; Thieme: Stuttgart, 1989; Vol. E19, pp 1568-71.

(5) (a) Fields, E. K. *J. Am. Chem. Soc.* **1962**, *84*, 1744-5. (b) Krapcho, A. P. *J. Org. Chem.* **1962**, *27*, 2375-7. (c) Kung, F. E.; Bissinger, W. E. *Ibid.* **1964**, *29*, 2739-42. (d) Anderson, J. C.; Lindsay, D. G.; Reese, C. B. *J. Chem. Soc.* **1964**, 4874-9. (e) Seyferth, D.; Burlitch, J. M.; Minasz, R. J.; Mui, J. Y.-P.; Simmons, H. D., Jr.; Treiber, A. J. H.; Dowd, S. R. *J. Am. Chem. Soc.* **1965**, *87*, 4259-70. (f) Dimroth, K.; Kinzebach, W.; Soyka, M. *Chem. Ber.* **1966**, *99*, 2351-60. (g) Landgrebe, J. A.; Thurman, D. E. *J. Am. Chem. Soc.* **1969**, *91*, 1759-66. (h) Tabushi, I.; Yoshida, Z.; Takahashi, N. *Ibid.* **1970**, *92*, 6670-2. (i) Seyferth, D.; Washburne, S. S.; Attridge, C. J.; Yamamoto, K. *Ibid.* **1970**, *92*, 4405-17. (j) Seyferth, D.; Burlitch, J. M.; Yamamoto, K.; Washburne, S. S.; Attridge, C. J. *J. Org. Chem.* **1970**, *35*, 1989-93. (k) Seyferth, D.; Mai, V. A.; Gordon, M. E. *Ibid.* 1993-8. (l) Dehmlow, E. V. *Tetrahedron* **1971**, *27*, 4071-5. (m) Seyferth, D.; Shih, H.-M.; Mazerolles, P.; Lesbre, M.; Joanny, M. *J. Organomet. Chem.* **1971**, *29*, 371-83. (n) Makosza, M.; Fedorynski, M. *Roczn. Chem.* **1972**, *46*, 311-3. (o) Tabushi, I.; Aoyama, Y.; Takahashi, M.; Gund, T. M.; Schleyer, P. v. R. *Tetrahedron Lett.* **1973**, 107-10. (p) Goh, S.-H.; Chan, K.-C.; Kam, T.-S.; Chong, H.-L. *Aust. J. Chem.* **1975**, *28*, 381-4. (q) Steinbeck, K. *Chem. Ber.* **1979**, *112*, 2402-12. (r) Harada, T.; Akiba, E.; Oku, A. *J. Am. Chem. Soc.* **1983**, *105*, 2771-6. (s) Hosomi, A.; Mikami, M.; Sakurai, H. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2784-94. (t) Ibatullin, U. G.; Petrushina, T. F.; Gataullin, R. R.; Safarov, M. G. *Khim. Geterotsikl. Soedin., Engl. Ed.* **1986**, *22*, 147-8. (u) Brinker, U. H.; Arct, J. Unpublished results, 1987. (v) Paquette, L. A.; Kobayashi, T.; Gallucci, J. C. *J. Am. Chem. Soc.* **1988**, *110*, 1305-7. (w) Rodin, A. P.; Nazarov, D. V.; Safiev, O. G. *Zh. Org. Khim., Engl. Ed.* **1990**, *26*, 852-4. (x) Likhotvorik, I. R.; Yuan, K.; Brown, D. W.; Krasutsky, P. A.; Smyth, N.; Jones, M., Jr. Unpublished results, 1991.

(6) For literature on reactions of carbenes with bicyclo[1.1.0]butanes and other strained compounds containing three-membered rings, see: Xu, L.; Miebach, T.; Smith, W. B.; Brinker, U. H. *Tetrahedron Lett.* **1991**, *32*, 4461-4 and references therein.

(7) When investigating reactions of bicyclo[2.1.0]pentane with dicarbomethoxycarbene and phenylcarbene generated by photolysis of the corresponding diazo precursors, Jones et al. found exo:endo isomers resulting from C-H insertions (ratios: ca. 1:2.7 and 1:2.4). With difluorocarbene, however, no C-H insertion product was found. See: Shiue, G.-H.; Misslitz, U.; Ding, X.; Jones, M., Jr.; de Meijere, A. *Tetrahedron Lett.* **1985**, *26*, 5399-402.

(8) Regen, S. L.; Singh, A. *J. Org. Chem.* **1982**, *47*, 1587-8.

(9) (a) Xu, L.; Tao, F.; Yu, T. *Acta Chim. Sinica, Engl. Ed.* **1987**, 294-8. (b) Xu, L.; Tao, F.; Yu, T. *Acta Chim. Sinica, Chin. Ed.* **1988**, *46*, 608-11; *Chem. Abstr.* **1988**, *109*, 189862r. (c) Xu, L.; Tao, F. *Synth. Commun.* **1988**, 2117-21.

(10) Friedrich, E. C.; Domek, J. M.; Pong, R. Y. *J. Org. Chem.* **1985**, *50*, 4640-1.

0002-7863/92/1514-783$03.00/0