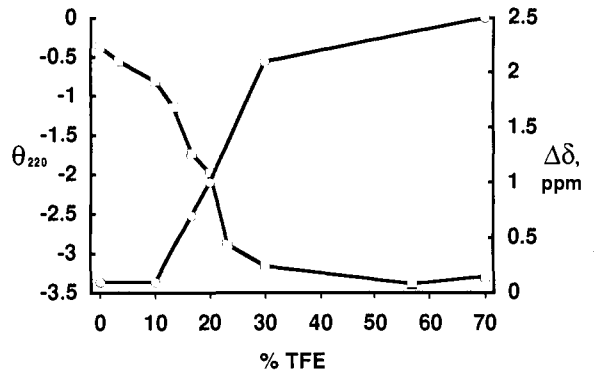

Figure 3. Plot of mean residue ellipticity at 220 nm, Θ_{220}, and secondary shift of the Val-10 Cα, $\Delta\delta$ (ppm), as a function of 2,2,2-trifluoroethanol (TFE) concentration in volume percent.

do not provide site-specific secondary structure information. In contrast, the effect of similar amounts of added TFE on the Cα chemical shifts is more revealing. Figure 2 shows the sequence-specific deviation of the Cα chemical shifts from their random coil values[7] (secondary shifts) at 10%, 20%, and 30% TFE. These histograms clearly indicate that the region from residue 6 to residue 12 is the most affected as the TFE concentration is increased from 10 to 30%. Residues 1-5 show little secondary shift. Large secondary shifts are observed starting at residue 6 and continuing through Val-10, with a somewhat smaller effect at Asn-6. The large secondary shifts are lost at Gly-11 but return at His-12. The last two residues exhibit only small effects. Analysis of these data suggests the formation of a helix between Asn-6 and His-12 which is interrupted by Gly-11.[8] While residues 13 and 14 may also be included in the helix, the smaller induced shifts suggest that these residues are at least partially unordered, possibly as a result of fraying at the peptide's C terminus. A fraying mechanism may also explain the smaller secondary shift observed for Asn-6. This description of the structure of bombesin is similar to that proposed by Carver and Collins[5] on the basis of NOEs and $^3J_{NH,H\alpha}$ measured in 70% TFE.

Comparison of the CD and the secondary shift results (Figure 3) indicates the cooperative formation of a helix with an inflection near 20% TFE and which is nearly complete at 50% TFE. This is readily explained by a two-state coil-to-helix equilibrium mediated by TFE concentration. At the highest TFE concentrations, the magnitude of the largest secondary shifts (2.0-2.5 ppm) is consistent with reported values for stable helices in proteins,[3] indicating that the equilibrium is far to the right and that a stable helix is formed.

Using bombesin as an example, we have shown that the Cα chemical shifts are very sensitive to helical secondary structure in small peptides as previously observed in proteins.[3] There is good agreement between the relative magnitudes of the secondary Cα shifts and the Θ_{220} determined from the CD measurements. This implies that an estimate of the population of the helical conformer can be extracted from the magnitudes of the Cα secondary chemical shifts. Furthermore, only the residues involved in helix formation are affected. Thus, unlike CD measurements, the ^{13}C secondary shifts are indicative of both the localization and the relative amounts of helical content. In systems which contain other types of secondary structures, such as β-sheet, coexisting with helical and random coil forms, two different possibilities arise. In the situation where β-sheet and α-helical structures are in equilibrium, the secondary shifts would be additive. Since the Cα secondary shifts tend to be opposite in sign for β-sheets than for α-helices,[3] this could result in a cancellation of the observed secondary shifts. On the other hand, if β-sheet and helical structures existed in distinct domains, these regions should be clearly defined by this method. Although not enough is known about the origin of the secondary shift effects to allow detailed quantification of helical content or the extent of coil-helix equilibrium, the method described here is clearly useful for monitoring transient helix formation in dynamic systems such as small peptides.

Supplementary Material Available: Tabulated ^{13}C chemical shifts for the α carbons of bombesin at 0, 10, 18, 20, 30, and 70% TFE in ^2H_2O (1 page). Ordering information is given on any current masthead page.

(7) In this study, the random coil chemical shifts for the Cα carbons were taken from the observed values for bombesin dissolved in 100% ^2H_2O solution, where bombesin is known to exist as a random coil (see refs 5 and 6). A similar analysis of the data using random coil shift values previously reported (Howarth, O. W.; Lilley, D. M. J. *Prog. NMR Spectrosc.* **1978**, *12*, 1-40) gave similar results.

(8) The smaller secondary shift of Gly-11 may be due to disruption of the helix, since glycine is a known "helix-breaker"; e.g., see Presta, L. G.; Rose, G. D. *Science* **1988**, *240*, 1632-1641. Another possibility is that glycine may have different secondary structure induced shifts than other amino acids.

Ring Fusion and Polycyclic Ring Constructions via Half-Open Titanocenes

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The construction of organic ring systems is of great importance. Such species are especially common in natural products.[3] While many synthetic routes are available for both small (3-6) and large (>10) ring systems,[4] approaches to intermediate-sized rings encounter serious problems as a result of entropic difficulties and severe transannular strain.[5] We report here on the coupling reactions of a (pentadienyl)titanium compound with acetylenes, which lead to fused and polycyclic systems that incorporate such medium-sized rings.[6] These reactions tolerate somewhat surprising functionalization and offer some potential for actual synthetic applications. Furthermore, subsequent carbon-carbon skeletal rearrangements can be promoted, and directed, by appropriate ligand substituents.

The reaction of $Ti(C_5H_5)(2,4\text{-}C_7H_{11})(PEt_3)$[7] ($C_7H_{11}$ = dimethylpentadienyl) with $C_6H_5C_2SiMe_3$ leads to the incorporation of 2 equiv of alkyne and the formation of a (bicyclo[4.2.1]nonadienyl)titanium complex (I, Scheme I, Figure 1a) in 64% yield. Attempts to limit the incorporation of alkyne, or to observe intermediates, were unsuccessful. Previous results suggest, however, that the first acetylene (C(6), C(7)) should couple to the pentadienyl ends (C(1,5)), leading to a seven-membered ring which rapidly undergoes further reactions of notably selective regiochemistry.[7,8] The product (I) may be considered to be a 16-electron complex by virtue of an apparent "agostic" interaction

(1) University of Utah.
(2) University of Delaware.
(3) Corey, E. J.; Cheng, X.-M. *The Logic of Chemical Synthesis*; John Wiley & Sons, New York, 1989; p 39.
(4) Mandolini, L. *Adv. Phys. Org. Chem.* **1986**, *22*, 1.
(5) Deslongchamps, P. *Aldrichimica Acta* **1984**, *17*, 59.
(6) (a) Taken in part from the Ph.D. Thesis of Dr. T. E. Waldman, University of Utah, 1990. A related photochemical nine-membered ring construction has also been reported with dienes.[6b] (b) Kreiter, C. G.; Lehr, K.; Leyendecker, M.; Sheldrick, W. S.; Exner, R. *Chem. Ber.* **1991**, *124*, 3.
(7) Melendez, E.; Arif, A. M.; Ziegler, M. L.; Ernst, R. D. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1099.
(8) (a) Kralik, M. S.; Rheingold, A. L.; Ernst, R. D. *J. Am. Chem. Soc.* **1987**, *6*, 2612. (b) Waldman, T. E.; Wilson, A. M.; Rheingold, A. L.; Melendez, E.; Ernst, R. D. *Organometallics*, in press. (c) Some mechanistic discussion may be found in ref 6a. Compound I could be viewed in part as an intermediate in β-hydride elimination.

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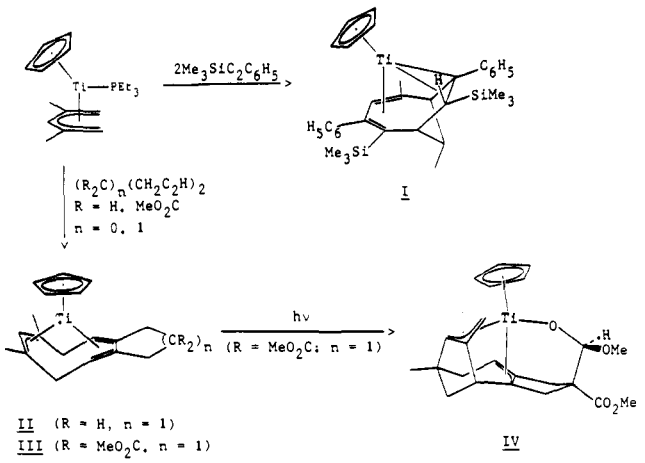
Scheme I



involving the C(8)–H bond (^{1}H NMR δ 0.24; Ti–C(8) = 2.449 (8) Å, cf., Ti–C(1,3,9) = 2.663 (8), 2.620 (8), 2.147 (8) Å), which is accompanied by a Ti–C(9) σ bond and an (η^4-diene)Ti interaction (C(4)–C(7)).

As the above results indicated that 2 equiv of an alkyne were optimal for these reactions, we utilized α,ω-diynes, and for 1,6-heptadiyne, a single, deep green product of the expected stoichiometry was obtained in good yield (Scheme I). The presence of some symmetry was revealed by NMR spectroscopy, suggesting structure **II**,[9] which was confirmed by X-ray diffraction (Figure 1b). As can be seen, an interesting [7.3.0] fused ring system has been constructed, in which a nine-membered ring is present, as a result of the coupling of the pentadienyl and diyne termini. Of notable interest is the fact that longer α,ω-diynes yield similar green products,[10] indicating the likelihood that other sized rings may also readily be fused to the nine-membered ring. Even more surprising, a similar, fully characterized product results from the corresponding reaction with hexadiyne,[11] so that it is possible to fuse a strained nine-membered ring to a severely strained four-membered ring (as occurs for caryophyllene[12]).

Of further significance is the fact that a similar reaction with $(MeO_2C)_2C(CH_2C_2H)_2$ led to a high yield of a nearly spectroscopically identical product, **III** (Scheme I). Surprisingly, then, the oxophilic titanium center has selectively attacked the alkyne in preference to the oxygen atoms. Thus, these reactions tolerate significant functionalization. Interestingly, the green complex **III** (and some of the other coupling products) undergoes a slow transformation on standing in solution, yielding an unsymmetric species. The reaction for **III** is reasonably well-behaved and can be promoted photolytically, after which a red product can be isolated in high yield. Spectral data were inconsistent with any simple transformation, and a structural determination revealed that a remarkable rearrangement of the skeletal carbon atoms had occurred, leading to the tricyclic [7.2.1.0^{2,6}] titanium complex **IV** (Scheme I, Figure 1c), essentially a $Ti(C_5H_5)(allyl)_2(OR)$ species. Perhaps the easiest way to envision the rearrangement process would involve breaking the C(7)–C(8) bond (referring to **II**'s skeleton in Figure 1b), while forming bonds between C(8) and C(17) and between C(7) and C(9). The alternative process, in which the C(9)–C(10) bond is broken while the C(8)–C(10) and C(9)–C(17) bonds are formed, appears less likely but probably cannot be eliminated from consideration without an isotopic labeling study. In addition, a Ti–O bond is formed in the process, and hydrogen atoms also undergo rearrangements, particularly to C(22). Of course, an entirely different rearrangement must

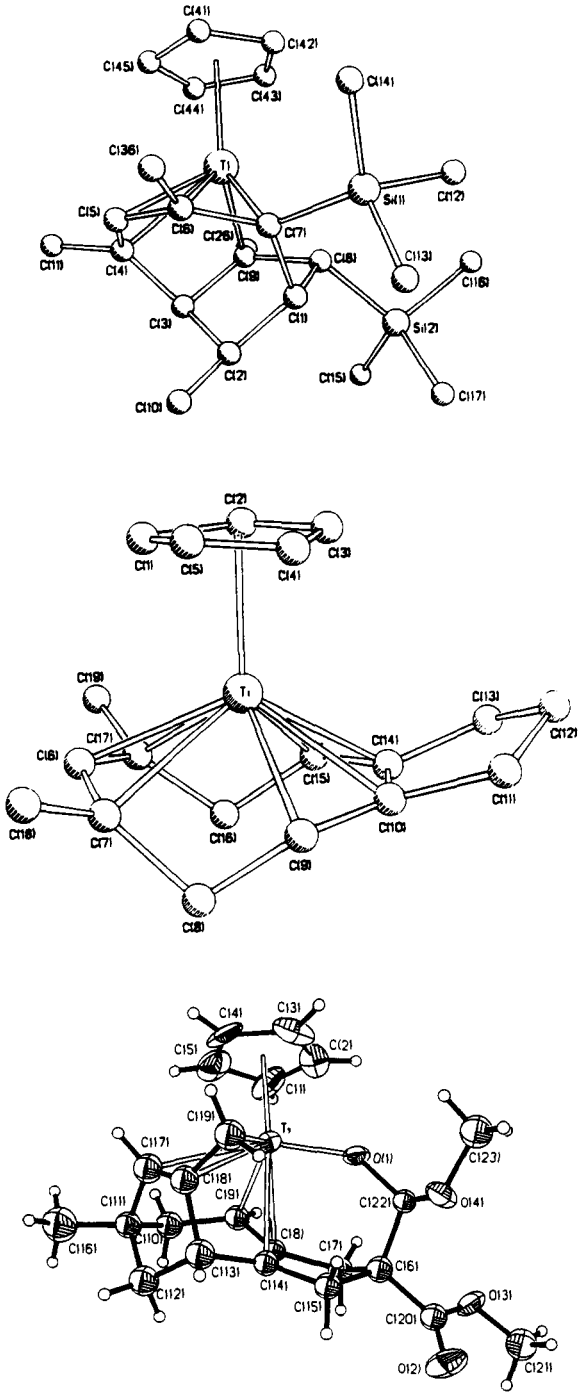


Figure 1. Perspective views of $Ti(C_5H_5)(2,4\text{-}C_7H_{11})$–alkyne coupling products (a) with $C_6H_5C_2SiMe_3$ (only the ipso carbon atoms (C(26), C(36)) of the phenyl groups are shown for clarity), (b) with 1,6-heptadiyne, and (c) with $(MeO_2C)_2C(CH_2C_2H)_2$ after subsequent rearrangement.

be followed by **II**. While possibly coincidental, both **I** and **IV** contain a [4.2.1] bicyclic fragment. These and other mechanistic points will be addressed in the future,[8c,13] in addition to efforts to remove and isolate the organic fragments (which have already succeeded for some coupling products).[14]

There seem to be many promising variations of these reactions to explore. Some rearrangements are quite substituent driven (for both the pentadienyl and diynes), and the diyne length, cyclopentadienyl substituents, and utilization of bridged pentadienyl (e.g., cyclohexadienyl) or other acyclic ligands could also lead to

(9) The framework differences between **I** and **II** may result from the greater steric bulk of $C_6H_5C_2SiMe_3$, which impedes additional coordination long enough for both alkyne ends to couple to the dienyl ends, or from the proximity of a second alkyne in the diyne reaction.

(10) Wilson, A. M.; Ernst, R. D. Unpublished results.

(11) See the supplementary material.

(12) *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: New York, 1989; Vol. 3.

(13) Whether the conversion involves a di-π-methane rearrangement, or is more metal dependent, is not clear.

(14) Wilson, A. M.; West, F.; Ernst, R. D. Unpublished results.

major interesting differences. These possibilities are under continuing investigation.

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Supplementary Material Available: Tables of bonding and thermal parameters, positional coordinates, and synthetic and spectroscopic details for the reported compounds (37 pages); tables of observed and calculated structure factors (41 pages). Ordering information is given on any current masthead page.

Oligodeoxynucleotides That Contain 2′,5″ Linkages: Synthesis and Hybridization Properties

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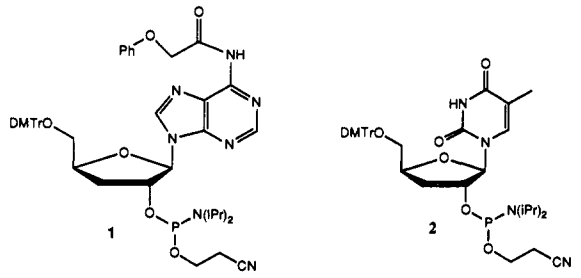
Would DNA be as useful a genetic material if the 3′-oxygen had been removed from RNA, forcing a 2′,5″ link? Divergent predictions have been offered,[1-6] but there was no experimental evidence on the properties of 2′,5″ DNA.[7,8] Our molecular modeling confirms that antiparallel Watson–Crick base pairing is possible, but in an open helix with poorer than normal base stacking. We have now prepared 16-mer oligodeoxynucleotides that contain one or more 2′,5″ linkages. Such linkages prove to destabilize normal 3′,5″ double helices. There is at best weak strand association, and that only at high salt concentrations, in isomeric DNA in which *all* links are 2′,5″.

We have described the synthesis of **1** and **2**.[9] Oligonucleotide synthesis was carried out on an Applied Biosystems Model 381A DNA synthesizer. The manufacturer's cycles and reagents proved satisfactory for coupling using phosphoramidites **1** and **2**. Since



the manufacturer's columns were used, all oligonucleotides, including those that contain only 2′,5″ linkages, have a (irrelevant) terminal 3″-hydroxyl. Deprotection, and purification by denaturing PAGE, followed standard protocols. Compounds **11**, **12**, **14**, and **16** were examined by electrospray mass spectroscopy and showed the expected negative molecular ions.

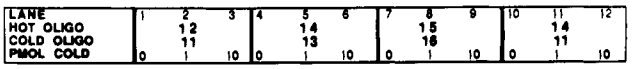


Figure 1. Native polyacrylamide gel (20%, 1× TBE). Oligomers **12**, **14**, and **15** were 5′-end-labelled with γ-^{32}P ATP and T4 polynucleotide kinase. The labeled oligomer ("hot") (**12** lanes 1–3, **15** lanes 7–9, **14** lanes 4–6 and 10–12, <0.1 pmol each) was combined with unlabeled oligomer ("cold") (**11** lanes 2, 3, 11, and 12, **13** lanes 5 and 6, **16** lanes 8 and 9, 1 pmol in lanes 2, 5, 8, and 11, 10 pmol in lanes 3, 6, 9, and 12) in 6 mL of 5% glycerol/0.1 M load buffer, heated to 37 °C, slowly cooled to 0 °C, loaded, and run at 5 °C.

Table I[a]

Nucleotide		Sequence		Tm
ANTI-16(**3**)	3′	TCCGTACGTTCGAACA	5′	
NORMAL-16(**4**)	5′	AGGCATGCAAGCTTGT	3′	65.0
(**5**)		AGGCATGC**A**AGCTTGT		54.0
(**6**)		AGGC**A**TGCA**A**GCTTGT		52.9
(**7**)		AGGCATGC**AA**GCTTGT		53.7
(**8**)		AGGCA**T**GCAAGCTTGT		60.1
(**9**)		AGGCATG**T**AAGCTTGT		50.9
(**10**)		AGGCATGC**C**AGCTTGT		50.7
(**11**)	5′	**AATAATAAATAATAA**T	3′	
(**12**)	3′	T**TATTATTTATTATTA**	5′	
(**13**)	5′	AATAATAAATAATAAT	3′	
(**14**)	3′	TTATTATTTATTATTA	5′	
(**15**)	5′	**AAAAAAAAAAAAAAA**T	3′	
(**16**)	3′	T**TTTTTTTTTTTTTTT**	5′	
(**17**)	5′	**AAAAAAAAAAAAAAA**A	3′	

[a] ***A*** and ***T*** designate the 2′,5′ isomer. Compounds **5**–**10** have the same sequence as oligomer **4** except where underlined. T_m's were measured at oligomer concentrations of 5–10 μM in 1× SSC (150 mM sodium chloride, 15 mM sodium citrate, pH 7.29), to which Na_3EDTA was added to 1 mM. Absorbance changes (260 nm) were monitored on a Cary 3 UV spectrometer, heating at 1 deg/min.

For T_m studies,[10] equimolar concentrations (estimated from calculated extinction coefficients) of oligomer **3** and complements **4**–**10** were combined in 1× SSC (0.15 M NaCl, 15 mM sodium citrate). As shown in Table I, replacement of one 3′,5″ linkage (in **4**) by a 2′,5″ connection (in **5**) destabilizes the duplex with **3** nearly as much as a pyrimidine–pyrimidine mismatch in oligomer **10**. The potentially wobble-paired **9** is nevertheless destabilized. The subtlety of the effects of this substitution is clear from the lack of much extra destabilization in doubly altered **6** and **7** or the small effect seen in **8**.

When the fully 2′,5″-linked oligomer combinations **11** and **12** or **16** and **17** (1 μM each) are heated from 2 to 82 °C in 1× SSC, a steady increase in absorbance (260 nm) is observed but no cooperative transitions are detectable. Two increasingly deep cooperative transitions appear for **16**/**17** with 0.5 or 1.0 M NaCl, centered at 7 and 32 °C (0.5 M) and 8 and 35 °C (1.0 M), but none for **11**/**12**. Confirming the lack of association in **11**/**12**, the circular dichroism of the combination (5 μM each, 1 M NaCl, 15 mM sodium citrate, 19 °C) was identical to the summed CDs of the components; by contrast, **13**/**14** gave a large incremental CD under these conditions, as did **16**/**17** (2 μM each).

(1) (a) Lohrmann, R.; Orgel, L. E. *Tetrahedron* **1978**, *34*, 853–855. (b) Sulston, J.; Lohrmann, R.; Orgel, L. E.; Schneider-Bernloehr, H.; Weimann, B. J.; Miles, H. T. *J. Mol. Biol.* **1969**, *40*, 227–234.

(2) Usher, D. A.; McHale, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1149–1153. Note that the high hydrolytic reactivity of the 2′,5″ linkage seen here occurred when it was part of a normal 3′,5″ oligonucleotide.

(3) (a) Srinivasan, A. R.; Olson, W. K. *Nucleic Acids Res.* **1986**, *14*, 5461–5479. (b) Dhingra, M. M.; Sarma, R. S. *Nature* **1978**, *272*, 798–801.

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(7) Hashimoto, H.; Switzer, C. *J. Am. Chem. Soc.*, in press. Note that the self-complementary oligomer proved to be monomolecular.

(8) Kierzek, R.; He, L.; Turner, D. H. *Nucleic Acids Res.* **1992**, *20*, 1685–1690.

(9) Rizzo, C.; Dougherty, J.; Breslow, R. *Tetrahedron Lett.*, in press.

(10) Puglisi, J. D.; Tinoco, I., Jr. In *Methods in Enzymology*; Dahlberg, J. E., Abelson, J. N., Eds.; Academic Press, Inc.: New York, 1989; Vol. 180, pp 304–325.

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