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Synthesis and chemistry of chromoprotein antitumor antibiotics: Nine-membered enediynes are equilibrated with p-benzyne type biradicals

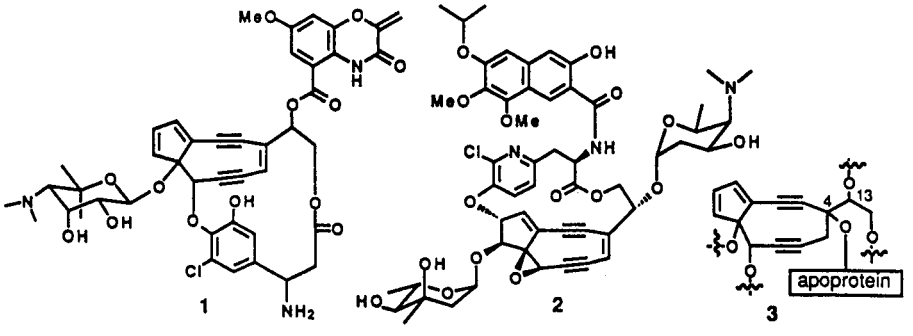
Masahiro Hirama

Department of Chemistry, Graduate School of Science, Tohoku University, Sendai 980-77, Japan

Abstract: The recent discovery of nine-membered cyclic enediyne chromophores (**1** for the antitumor antibiotics C-1027; **2** for kedarcidin) stabilized by specific apoproteins prompted us to synthesize a highly strained carbocyclic core structure to elucidate the specific mechanism which prevents their spontaneous aromatization. We have achieved successful synthesis of enediynes **10** and **14** as models of **1** and **2**, respectively, and found the remarkable solvent dependence of the rate of cycloaromatization of **14**. The kinetic data and the ESR spectra strongly indicate that the hydrogen abstraction rate of p-benzyne biradical **17** is slower than that of phenyl radical by a factor of 100, and that the equilibrium is virtually reached between **14** and **17** in CH_3CN and CD_2Cl_2 at ambient temperature, which suggests a hypothesis that the chromophores **1** and **2** may also be equilibrated with their p-benzyne forms and are stabilized kinetically by specific apoproteins. Thus, those molecules may exist indefinitely if they remain free of hydrogen donor(s) in the holoprotein complex. The kinetics and energetics of Bergman cycloaromatization as well as our endeavours toward the total syntheses of **1** and **2** are disclosed.

INTRODUCTION

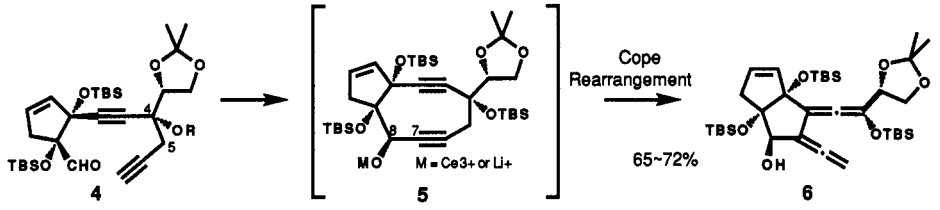
Very recently, chromophores of the potent antitumor antibiotic chromoprotein C-1027 (**1**)[1] and kedarcidin (**2**)[2] have been shown to possess a highly strained bicyclo[7.3.0]dodecadiyne core structure. Since such nine-membered 3-ene-1,5-diyne systems are highly labile to undergo cycloaromatization at ambient temperature,[1-3] a specific mechanism which prevents spontaneous aromatization of **1** and **2** should be identified in the holoprotein. Although some noncovalent stabilization interactions have been suggested between **1** and the apoprotein, they have not been verified.[1b,4] The most simple alternative might be to bond them covalently as a protein-conjugate **3**. This strategy, which masks the 3-ene-1,5-diyne system **1** as a 1,5-diyne **3**, is also a fascinating approach from the perspective of design and synthesis of related DNA-cleaving molecules.[3b,5] We describe here a general and efficient route to the highly strained bicyclo[7.3.0]dodecadiyne system, a fine tuning of the extremely facile Cope rearrangement of 9-membered cyclic 1,5-diynes,[6] and moreover the successful synthesis of enediynes, **10** and **14**, as models of **1** and **2**, respectively, as well as their equilibration with a p-benzyne biradical and the kinetic stabilization.[7]



SYNTHESIS OF NINE-MEMBERED CYCLIC 1,5-DIYNES

Synthesis and extremely facile Cope rearrangement

Several groups have recently succeeded in synthesizing the relevant 9-membered diynes. They used techniques to minimize the high enthalpic and entropic barriers, such as ring contraction,[3b,5,8] assembly of either a cis-epoxide[9a] or cis-olefin between the two acetylenic bonds, and bending of the acetylenic bond as a cobalt complex.[10] These results encouraged us to examine the straightforward construction of the bicyclo[7.3.0]dodecadiyne system through an intramolecular acetylide addition from a precursor such as **4**, which possesses a conformationally non-rigid C4-C5 single bond.

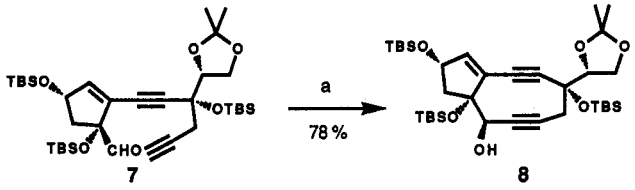


Scheme 1. $LiN(TMS)_2$ (6~23 mol eq), anhydrous $CeCl_3$ (7~20 mol eq), THF (3~6 mM), -30°C ~ rt, 30 min.

When cyclization of **4** was attempted by adding a large excess of lithium hexamethyldisilazide [$LiN(TMS)_2$, 20-30 eq] in THF at -50°C, only the C_2 symmetrical dimer was produced in 20-40% yield, even under high dilution conditions (2 mM). In the presence of anhydrous cerium chloride,[9] however, a monomeric product was formed at a higher temperature (-30°C ~ rt). Surprisingly, a bis-allene **6** was isolated as a single stereoisomer in a yield of up to 72%. Formation of the C7-C8 bond clearly indicated that the 9-membered cyclic diyne **5** acted as an intermediate, and was followed by Cope rearrangement (Scheme **1**).[6] Cope rearrangement of acyclic 1,5-diynes has been shown to occur above 200°C.[11] Thus, we found that the bicyclo[7.3.0]dodecenediyne system, such as in **5**, undergoes an extremely facile Cope rearrangement.[6]

Fine tuning of facile Cope rearrangement

We next examined how we could suppress the above rearrangement. Review of the relevant isolated systems[3b,5,9a] suggested that a common structural feature among them is that they contain a cyclopentene double bond exo to the 9-membered ring. Therefore, we synthesized **7** from **4**. Addition of **7** to the $LiN(TMS)_2/CeCl_3$ mixture at -40°C followed by stirring at room temperature for 1 h yielded a cyclic diyne **8** as a single stereoisomer at a yield of 78% without contamination of the corresponding bis-allene (Scheme 2). The product **8** is not stable at room temperature but can be stored in solution at -20°C without deterioration.[6]



Scheme 2. (a) $LiN(TMS)_2$ (10~23 eq), anhydrous $CeCl_3$ (11~25 mol eq), THF (1~2 mM), -40°C ~ rt, 1 h.

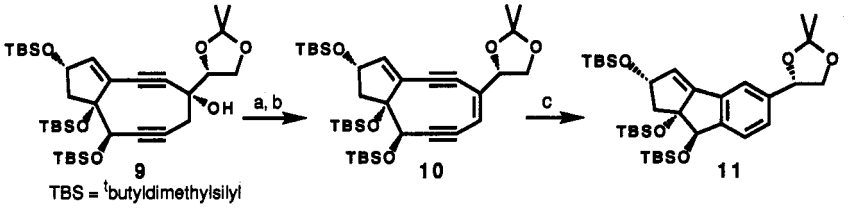
Cope rearrangement of **8** to the bis-allene took place in deoxygenated toluene-d_8 at a higher temperature. The half-life for rearrangement of **8** at 50°C is 6.4 h (^1H NMR analysis), which indicates that the rate of the Cope rearrangement can be modified by a small structural change, such as the shift of a double bond. Molecular mechanics calculations (CAChe, MM2) suggest that the transformation **5**→**6** is more exothermic, so that **5** would more readily undergo rearrangement than **8**.[6]

Previous syntheses of both cyclononadiyne and cyclodecadiyne rings related to enediyne antibiotics[12] via intramolecular acetylide additions were ensured by the presence of at least two structural elements which reduced the degree of conformational freedom of the substrate, i.e., cis-olefin (or cis-epoxide) and an additional 5- or 6-membered carbocyclic fused ring. In this study, we have demonstrated that $LiN(TMS)_2/CeCl_3$-mediated cyclization procedures[9] can be used to construct the highly strained cyclononadiyne system even if the former principal element is absent. The diyne **8** possesses the appropriate functionality to synthesize the chromophores of C-1027 (**1**)[1] and kedarcidin (**2**).[2]

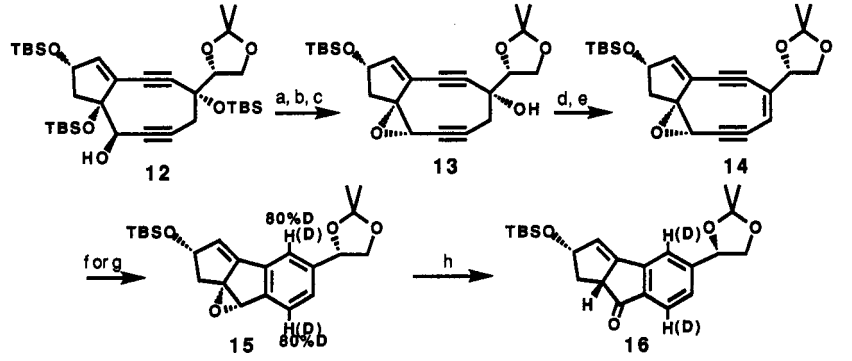
SYNTHESIS AND CHARACTERIZATION OF NINE-MEMBERED CYCLIC ENEDIYNES

Synthesis, isolation, and reactions

Nine-membered diynes **9** and **12** were converted to enediynes **10** and **14** as shown in Schemes 3 and 4, respectively.[7] The presence of an epoxide ring in the mesylate of **13** greatly facilitated the elimination reaction, which was completed within 30 min in the presence of DBU (~6 equiv) in CH_2Cl_2 at 25°C. This is approximately ten times faster than that of the mesylate of **9**. The enediyne **10** was too labile to be isolated as anticipated[1,3] and rapidly underwent spontaneous cycloaromatization ($t_{1/2}$ ~11 min) in the presence of excess 1,4-cyclohexadiene at room temperature to afford **11** in a good yield (~87%). On the other hand, the epoxy enediyne **14** was more stable and could be purified by silica gel chromatography. Cycloaromatization of pure **14** was approximately four times slower than that of **10** in THF-d_8. Quantitative formation of unstable **15** in 1,4-cyclohexadiene-CH_2Cl_2 (1:1) was confirmed by NMR, but removal of the solvent or silica gel chromatography resulted in its complete decomposition. In this case, ketone **16** was isolated as a major product (~14%) by GPC column filtration. Bis-deuterated **15**-d_2 and **16**-d_2 were produced in the perdeuterated solvents.[7]



Scheme 3. (a) MsCl, Et_3N, CH_2Cl_2; (b) DBU (~6 equiv), CH_2Cl_2, 25°C, 6 h; (c) 1,4-cyclohexadiene / CH_2Cl_2, 25°C, 87% from **9**.



Scheme 4. (a) MsCl, Et_3N, DMAP, CH_2Cl_2; (b) TBAF, THF, 0°C, 75% from **12**; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2, -78°C, 91%; (d) MsCl, Et_3N, CH_2Cl_2; (e) DBU (~6 equiv), CH_2Cl_2, 25°C, 0.5 h; (f) 1,4-cyclohexadiene / CH_2Cl_2, 25°C, ~100% from **13**; (g) THF-d_8, 27°C, 82%; (h) purification on GPC column, ~14%.

Remarkable solvent dependence of rate of cycloaromatization

Yoshida and co-workers recently observed the unexpected solvent-dependence of the rate of cycloaromatization of **1**.[13,14] Since a pure 9-membered enediyne **14** that is soluble in most organic solvents is now available, we examined more precisely the cycloaromatization rate of **14** in various solvents. We were again surprised that the pseudo first-order decay of **14** is highly dependent on the solvent as a hydrogen donor (Table 1).[7] The data showed the relative rates of THF, benzene, and CH_3CN to be 1:0.2:0.1, which were only slightly lower than those reported for hydrogen abstraction by phenyl radical (1:0.1:0.02).[15a] A primary kinetic isotope effect was also noticed: its magnitude increased as the reaction became slow, as has been observed generally in hydrogen transfer reactions.[16] These results indicate that the hydrogen abstraction step by a p-benzyne biradical intermediate[17,18] **17** is kinetically significant, or rate-limiting in the cycloaromatization of **14** (Scheme 5),[7] while the first cyclization step is known to be rate-determining for acyclic enediynes.[17,19]

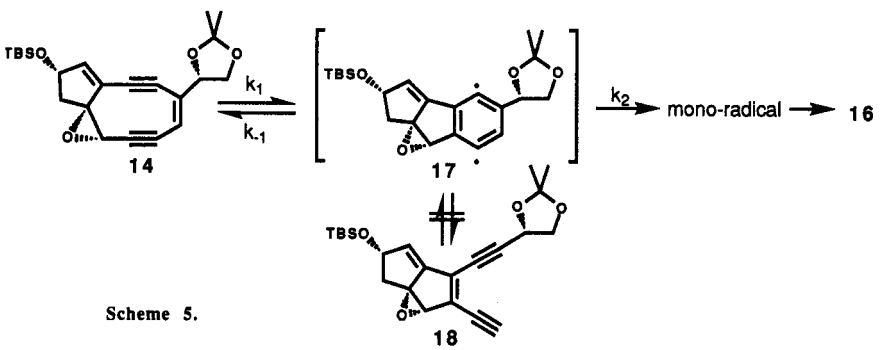
TABLE 1. Cycloaromatization Rate of **14** in Various Solvents at 28°C[a)]

Entry	Solvent	$t_{1/2}$ (min)[b)]	k (x 10^{-5} s^{-1})[b)]	Relative rate
1	CD_2Cl_2	680	1.7	0.035
2	CH_3CN	610	1.9	0.039
3	C_6H_6	330	3.5	0.071
4	1,4-dioxane-d_8	310	3.7	0.076
5	1,4-dioxane	110	11	0.22
6	THF-d_8[c)]	220	5.4	0.11
7	THF	68	17	0.35
8	CD_3CD_2OD	130	8.8	0.18
9	CH_3CH_2OH	65	18	0.37
10	1,4-C_6D_8/CD_2Cl_2[d)]	28	41	0.84
11	1,4-C_6H_8/CH_2Cl_2[e)]	23	49	1.0

a) Measured by HPLC except entry 6. b) Deviation : ± 1 ~ ± 6 % c) Measured by ^{1}H-NMR. d) 1,4-Cyclohexadiene-d_8 / CD_2Cl_2 = 1 / 1 (v/v). e) 1,4 Cyclohexadiene / CH_2Cl_2 = 1 / 1 (v/v).

Kinetics and energetics for cycloaromatization

Thus, nine-membered enediyne **14** may be virtually in equilibrium with p-benzyne biradical **17** in CH_3CN and CD_2Cl_2 in which cycloaromatization is substantially retarded. If the hydrogen abstraction rate of **17** is slower than that of phenyl radical (CH_3CN, k_H = 1.0 x 10^5 M^{-1} s^{-1})[15a] due to steric hindrance and/or through-bond interaction by a factor of about 100,[7,20] the equilibrium constant (K) in CH_3CN is estimated to be 2 x 10^{-9} (ΔG = ~12 kcal/mol) by steady state approximation to the concentration of **17** ($k_{obs} = k_1k_2/k_{-1} = Kk_2$) based on the assumption of the pseudo first order kinetic constant $k_2 = k_H[CH_3CN] \times 10^{-2} \approx k_H \times 10^{-1}$ s^{-1}. This ΔG seems not unreasonable because the sum of this value and an E_a for hydrogen abstraction by phenyl radical (4~7 kcal/mol)[15b] and the increment due to steric hindrance and/or through-bond interaction, ~3 kcal/mol, is in good agreement with an apparent activation energy for the decay of **14** [E_a = 21.6 kcal/mol (ln A=28.5: 20~32°C in 1,4-C_6H_8/CH_2Cl_2); 18.5 kcal/mol (ln A = 21.9: 40~60°C in EtOH)] obtained by the Arrhenius plot of the rate constants. Thus, the hydrogen abstraction rate of p-benzyne type biradical **17** is most likely to be slower than that of phenyl radical by a factor of 100, contrary to the common sense.[7,20]

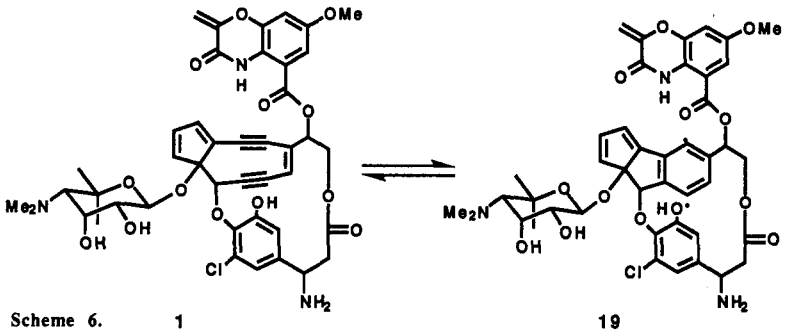


Scheme 5.

Equilibration of nine-membered enediyne with p-benzyne

The ΔG (~12 kcal/mol) is similar to that reported for acyclic systems.[17,18a] It suggests that energy of **17** may also be raised to such an extent that **14** is destabilized by nine-membered ring strain. The destabilization should arise from the presence of a 1,8-double bond and an epoxide ring in dehydrobenzopentalene core of **17**,[6] as indicated by MM2 calculations.[7] The barriers for cycloaromatization of acyclic (*Z*)-3,4-dipropylhex-3-ene-1,5-diyne (**19**) to 2,3-dipropyl-1,4-dehydrobenzene (**20**), its back reaction, and an alternative ring opening of **20** to (*Z*)-dodec-6-ene-4,8-diyne (**21**) were reported to be 27.4, ~16, and ~10 kcal/mol, respectively.[17b] Therefore, the destabilization of both **14** and **17** should significantly decrease the barrier for their interconversion and consequently the process became reversible at ambient temperature. The above Bergman's observation[17b] and our failure to

detect even trace amounts of an acyclic enediyne **18**[13] strongly suggest that cleavage of the less substituted bond of **17** leading to **18** has a considerably higher barrier.[7] Nine-membered ring constraint may also contribute to lowering the barrier to **14** because the reaction of **17** to **14** requires less molecular deformation. Thus, the kinetic significance of hydrogen abstraction step in the present system may be attributable to the large energy difference between **14** and **17**, i.e., the extremely low concentration of **17**, and the very low barrier for back reaction to **14**, and the slower hydrogen abstraction by p-benzyne **17**.[7]



Scheme 6. **1** **19**

Kinetic stabilization of natural chromoprotein antibiotics

These observations for **14** suggest a hypothesis that the chromophores (**1** and **2**) may also be equilibrated with their p-benzyne form and are stabilized kinetically by a specific apoprotein. Thus, **1** and **2** may exist indefinitely if they remain free of hydrogen donor(s) in the holoprotein complex.[7] Aromatic amino acid residues, such as Tyr32, as well as the internal tyrosine and benzoxazine rings of **1**, seem to play an important role in this kinetic stabilization.[21] Our observation of p-benzyne biradicals **17** and **19** in equilibrium by ESR spectroscopy should support the above hypothesis of kinetic stabilization.[7,22]

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21. Our NMR and computer modeling studies indicated that Tyr32 is proximate to the nine-membered core of **1** (Iida, K.; Fukuda, S.; Tanaka, T.; Hirama, M.; Imajo, S.; Ishiguro, M.; Yoshida, K.; Otani, T. *Tetrahedron Lett.* in press). For another modeling study, see: Okuno, Y.; Otsuka, M.; Sugiura, Y. *J. Med. Chem.* **1994**, *37*, 2266, and also see ref 4.
22. Preliminary ESR measurements for a CD_2Cl_2 (or CH_3CN) solution of **14** and the powder C-1027 holoprotein exhibited broad spectra whose intensity varied reversibly with temperature. Identification and analysis of these spectra will be reported in due course.