Table I. Experimentally Determined Average Molecular Weight (M_r) for the Native and Reduced Forms of the Proteins Bovine Proinsulin and α-Lactalbumin after Reaction with D_2O, % H/D Exchange, and $\Delta N/\Delta R$ (the Ratio of the Difference in Molecular Weight after Reaction with D_2O for the Native and Reduced Forms of the Protein)

	M_r after D_2O reaction	% H/D exchange	$\Delta N/\Delta R$
native proinsulin (120 °C)[a]	8703.8 ± 1.4	16.7	1.87 ± 0.25
reduced proinsulin (120 °C)[b]	8699.6 ± 1.4	8.9	
native proinsulin (145 °C)[a]	8715.0 ± 1.4	25.2	1.91 ± 0.25
reduced proinsulin (145 °C)[b]	8705.2 ± 1.4	12.6	
native α-lactalbumin (120 °C)[c]	14277.0 ± 2.2	44.8	1.18 ± 0.06
reduced α-lactalbumin (120 °C)[d]	14269.6 ± 2.2	36.9	

[a] M_r = 8681.8. [b] M_r = 8687.8. [c] M_r = 14175.2. [d] M_r = 14183.0.

accounting for the M_r change due to the reduction of three disulfide bonds.

Comparison of H/D exchange observed for the native and reduced protein ions of the same charge state shows that ions formed from the native protein are more reactive than those from the reduced form. The charge-state distribution observed in the ESI m/z spectrum for native proinsulin consists primarily of the 7^+ and 8^+ charge states, while the distribution observed for the reduced proinsulin ranges from 7^+ to 11^+. Upon reaction with D_2O (or H_2O), the only charge state observed for the native protein is 7^+, while the charge states 7^+ to 9^+ are observed for the reduced protein.[12] While it is not possible to determine whether the extent of H/D exchange differs for the two initial charge states of the native protein, no significant difference is observed for the limited number of product charge states after reaction of the reduced protein.

To investigate whether $\Delta N/\Delta R$ is also dependent upon the reaction temperature, the same reactions were performed at 120 °C, and the value of $\Delta N/\Delta R$ was determined to be 1.87 ± 0.25. This suggests that, unlike the total extent of H/D exchange, the relative extent of H/D exchange rate is not strongly temperature-dependent over the range subject to investigation (typically 90–150 °C).[13] Table I summarizes these results as well as those obtained for ions formed from the native and reduced forms of α-lactalbumin at 120 °C where a smaller but still significant $\Delta N/\Delta R$ ratio of 1.18 ± 0.06 was observed.[10] These data show that gas-phase ions formed from these proteins exhibit opposite behavior toward H/D exchange than that observed in solution[5] where the denatured or reduced form is more reactive, presumably due to a more accessible solution structure.

The present results show that qualitative differences in the gas-phase structure of multiply charged macromolecules can be probed using high-pressure ion/molecule reactions. We speculate that the greater H/D exchange for the ions formed from native disulfide-bonded protein is due to Coulombic contributions[5,14,15] arising from the more compact gas-phase structure. Ogorzalek Loo et al. have recently reported that gas-phase proton-transfer reactions of multiply protonated proteins with diethylamine show higher reactivity with ions formed from the native protein as compared to ions from the reduced form having the same charge state,[16] a trend similar to that observed here. Possible Coulombic contributions to enhanced proton-transfer efficiency for highly charged ions have been noted.[3,4,7,15] In addition, Cooks and coworkers[17] have reported a dependency of H/D exchange rates on proton affinity differences in gas-phase ion/molecule reactions. It is reasonable that the gas-phase structure of the constrained disulfide-bonded protein will result in enhanced Coulombic effects compared to the disulfide reduced form. As a consequence, this increase in Coulombic energy may be sufficiently large to assist in overcoming reaction barriers, resulting in increased reactivity (e.g., H/D exchange, proton transfer, etc.) depending upon gas-phase structure and charge location.

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(12) The observed shift in the charge-state distribution is a result of proton-transfer reactions between multiply protonated protein ions and D_2O (or H_2O).[7]

(13) The minimum temperature is defined by the point below which substantial condensation of D_2O (or H_2O) occurs, resulting in loss of signal intensity due to extensive solvation and, at sufficiently low temperature, large clusters of droplets entering into the mass spectrometer. The upper limit is defined by the point above which thermally induced dissociation is prevalent. The actual temperature at which this occurs is dependent upon the specific protein,[9] but it is typically >150 °C for the experimental arrangement used in this work.

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(15) McLuckey, S. A.; Glish, G. L.; Van Berkel, G. J. *Proceedings of the 39th Conference Mass Spectrometry and Allied Topics*, Nashville, TN; ASMS: East Lansing, MI, 1991; p 901.

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(17) Ranasinghe, A.; Hand, O. W.; Sethi, S. K.; Eberlin, M. N.; Riederer, D. E.; Cooks, R. G. *Proceedings of the 39th Conference Mass Spectrometry and Allied Topics*, Nashville, TN; ASMS: East Lansing, MI, 1991; p 1631.

Application of the Allylic Diazene Rearrangement: Synthesis of the Enediyne-Bridged Tricyclic Core of Dynemicin A

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Since its isolation and structure determination, the enediyne-containing antibiotic dynemicin A (**1**) has stimulated much research.[1,2] Certainly one of the most demanding synthetic challenges posed by dynemicin A is the dense array of sensitive functionality present in the molecule's enediyne core. Reported herein are studies that have culminated in a concise synthesis of the fully functionalized A–C rings of **1**. The synthesis features a previously described transannular Diels–Alder polycyclization[3] coupled with a highly efficient allylic diazene rearrangement[4] to rapidly assemble the enediyne-bridged A–C ring system, which is then elaborated to the fully functionalized dynemicin A core structure **2**.

As previously reported,[3] macrolactonization of seco acid **3** leads to efficient formation of polycyclization product **4**.[5] Attempts

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(1) (a) Konishi, M.; Ohkuma, H.; Matsumoto, K.; Tsuno, T.; Kamei, H.; Miyaki, T.; Oki, T.; Kawaguchi, H.; VanDuyne, G. D.; Clardy, J. *J. Antibiot.* **1989**, *42*, 1449. (b) Konishi, M.; Ohkuma, H.; Tsuno, T.; Oki, T.; VanDuyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1990**, *112*, 3715.

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(3) Porco, J. A., Jr.; Schoenen, F. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 7410.

(4) For recent applications of the allylic diazene rearrangement in synthesis, see: (a) Corey, E. J.; Wess, G.; Xiang, Y. B.; Singh, A. K. *J. Am. Chem. Soc.* **1987**, *109*, 4717. (b) Myers, A. G.; Kukkola, P. J. *J. Am. Chem. Soc.* **1990**, *112*, 8208. (c) Myers, A. G.; Finney, N. S. *J. Am. Chem. Soc.* **1990**, *112*, 9641. (d) Corey, E. J.; Virgil, S. C. *J. Am. Chem. Soc.* **1990**, *112*, 6429.

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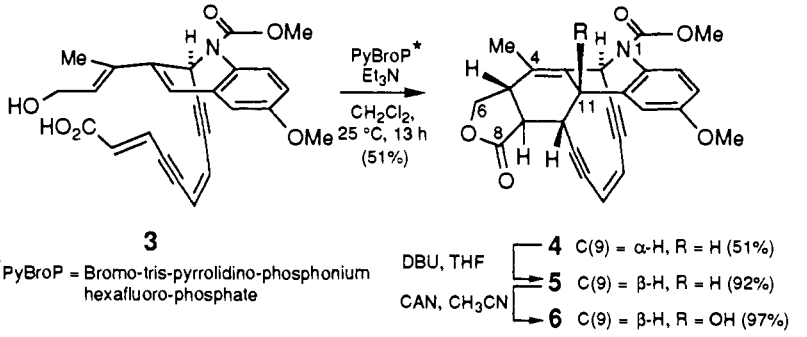
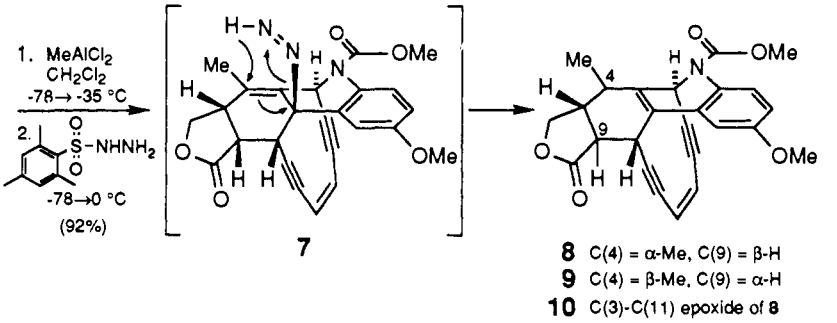
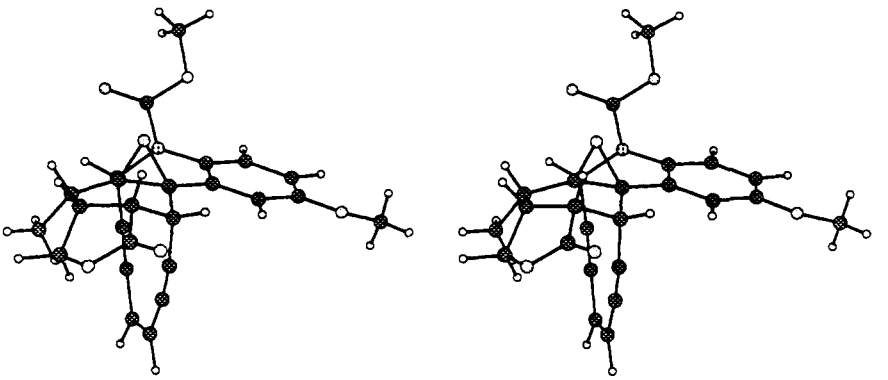
Scheme I

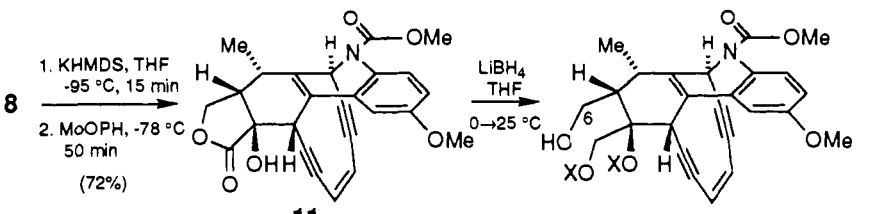


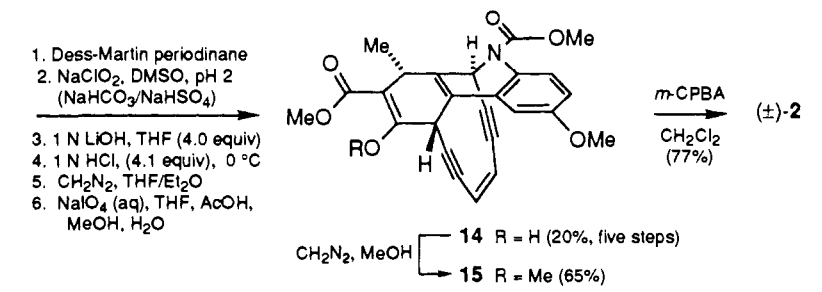


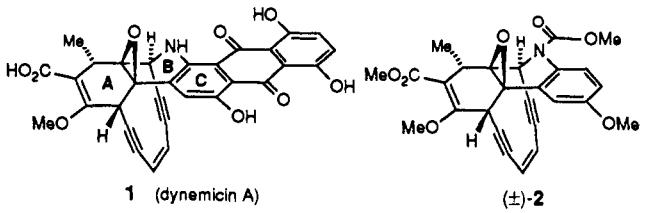


(Stereoview of epoxide **10**, Chem 3D Representation of X-ray Data)

Scheme II







1 (dynemicin A) (±)-2

Figure 1.

to simultaneously reposition the C(3)–C(4) olefin and set the C(4) methyl stereochemistry of **4** by applying an oxidation/reduction protocol [ceric ammonium nitrate (CAN), then $EtAlCl_2/Et_3SiH$] produced a product (**9**) possessing a transposed olefin, but incorrect methyl stereochemistry (Scheme I).[3] In our more recent investigations, we reasoned that increasing the convex/concave nature of the ring system might alter the stereochemical outcome of the Et_3SiH reduction. Hence, **4** was epimerized to *cis*-lactone **5**[6] (DBU, 25 °C, 14 h, 92% yield), which underwent benzylic oxidation with CAN to furnish alcohol **6**[6] as a single regioisomer (97% yield).[7] Surprisingly, **6** underwent $EtAlCl_2/Et_3SiH$-mediated reduction (−78 °C) to regenerate lactone **5** as the only isolable product. Thus, instead of altering the stereoselectivity of nucleophilic addition, epimerization to the *cis*-lactone results in an increased preference for attack at the benzylic position.

Although the increased propensity for nucleophilic addition to C(11) thwarted attempts at direct substitution by hydride at C(4), it suggested the possibility of utilizing an allylic diazene rearrangement as an alternative reduction strategy. After considerable experimentation, it was found that ionization of **6** ($MeAlCl_2$, CH_2Cl_2, −78 → −35 °C) and trapping of the presumed intermediate carbonium ion with mesitylenesulfonohydrazide[8] provided the desired isomer **8**[6] in 92% yield. A likely mechanism for this transformation involves a stereospecific [1,5] sigmatropic rearrangement of diazene **7**. The structure of **8** was secured by single-crystal X-ray analysis of **10**,[6] which resulted from epoxidation with *m*-CPBA (CH_2Cl_2, pH 7 buffer, 75% yield).

Having simultaneously established the methyl stereochemistry and correct positioning of the olefin for eventual epoxidation, we next focused our attention on developing an efficient protocol for conversion of the butyrolactone moiety in **8** to the vinylogous carbonate found in **1**. Toward this end, α-hydroxylation of **8** to **11**[6] (KHMDS, MoOPH, 72% yield) followed by lithium borohydride reduction delivered triol **12**,[6] which was selectively converted to the 1,2-cyclic carbonate **13**[6] (triphosgene, Et_3N, CH_2Cl_2, −78 °C).[9] Oxidation at C(6) and oxidative excision of C(8) were then performed via a five-step sequence without isolation of intermediates. Thus, sequential oxidation of **13** with the Dess–Martin periodinane and then aqueous sodium chlorite[10,11] furnished, after base/acid workup, the crude acid/carbonate which was, in turn, saponified (LiOH), esterified (CH_2N_2), and subjected to glycol cleavage ($NaIO_4$) to afford **14**[6] in 20% yield for the five steps.[12] Finally, etherification of **14** with CH_2N_2 in MeOH provided cyclohexadiene **15**,[6] which underwent selective epoxidation (*m*-CPBA, CH_2Cl_2, K_2HPO_4/KH_2PO_4 pH 7 buffer) to provide (±)-**2** (Scheme II).[6]

In summary, the combination of a transannular Diels–Alder polycyclization and an allylic diazene rearrangement provides a synthetic intermediate that can be efficiently elaborated to the enediyne-bridged tricyclic core of dynemicin A. Studies directed toward completion of the total synthesis are currently underway.

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Supplementary Material Available: Complete spectral data for compounds **2**, **5**, **6**, **8**, and **10**–**15** and crystallographic data for compound **10** (10 pages). Ordering information is given on any current masthead page.

(5) (a) Although the Yamaguchi macrolactonization protocol[5b] had been employed in preliminary studies,[3] improved yields were observed when the reaction was effected with PyBroP.[5c,d] The experimental simplicity and improved efficiency has currently led to routine use of PyBroP in large-scale cyclizations (e.g., 5 g of **3** were converted to 2.6 g of **5**). (b) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (c) Coste, J.; Frérot, E.; Jouin, P.; Castro, B. *Tetrahedron Lett.* **1991**, *32*, 1967. (d) Available from Novabiochem (cat. no. 01-62-0017), P.O. Box 12087, San Diego, CA 92112-4180.

(6) The structure assigned to each new compound is in accord with its infrared and high-field ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra, as well as appropriate parent ion identification by high-resolution mass spectrometry.

(7) Previous experiments in the *trans*-lactone series provided a mixture of the C(4)OH:C(11)OH regioisomers (1:3, respectively, 85% yield).

(8) Cusack, N. J.; Reese, C. B.; Risius, A. C.; Roozpeikar, B. *Tetrahedron* **1976**, *32*, 2157.

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(10) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277. This reaction is attended with a small amount of a byproduct (ca. 10%) which appears to be the C(3)-C(11) epoxide. However, this structure has yet to be fully delineated.

(11) (a) Dalcanale, E.; Montanari, F. *J. Org. Chem.* **1986**, *51*, 567. The use of DMSO as solvent and NaH_2PO_4 adjusted to pH 2 with concentrated HCl were required to minimize the formation of a byproduct, likely the C(3)–C(11) epoxide, which presumably derives from the presence of the $HOCl/Cl^-$ redox pair. (b) For the alternate use of 2-methyl-2-butene as an HOCl scavenger, see: Kraus, G. A.; Taschner, M. J. *J. Org. Chem.* **1980**, *45*, 1175 and references cited therein.

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Asymmetric α-Amination of Ketone Enolates by Chiral α-Chloro-α-nitroso Reagents: A New Approach to Optically Pure *erythro*-β-Amino Alcohols

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We have recently reported highly diastereoface selective C,N-bond formations in the reactions of 1-chloro-1-nitrosocyclohexane with chiral enolates.[1] More ambitiously, we envisaged the development of chiral α-chloro-α-nitroso reagents **V** capable of "aminating" prochiral ketone enolates **I** with high enantiofacial differentiation. Acid hydrolysis of the resulting nitrones **II** would provide optically pure β-keto *N*-hydroxylammonium salts **III** together with chiral ketone **IV**, which could be recycled to reagent **V** (Scheme I).

Oximation of known sulfonamides **1**[2] followed by chlorination furnished, after crystallization, pure blue chloro nitroso compounds **2a** (78% from **1a**) and **2b** (70% from **1b**, Scheme II). The structure of **2b**, as determined by X-ray diffraction,[3] is similar to that of (+)-10-bromo-2-chloro-2-nitrosocamphane.[4] Thus, the

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(3) Bernardinelli, G.; Oppolzer, W.; Sundarababu, G. *Acta Crystallogr.*, in press.

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