The First Total Synthesis of N1999-A2: Absolute Stereochemistry and Stereochemical Implications into DNA Cleavage

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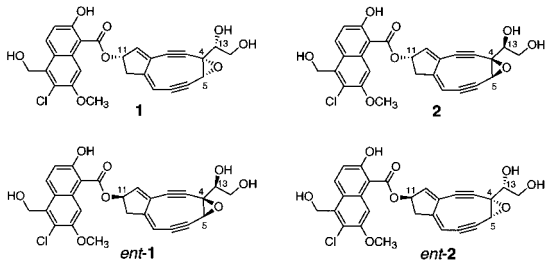
Enediyne antitumor antibiotics have attracted immense attention among chemists and biologists alike because of their unique chemical structures, potent antitumor activities, and fascinating biological modes of action.[1] As a novel addition to this family, the nonprotein and extremely strained nine-membered enediyne antibiotic N1999-A2 strongly inhibits the growth of various tumor cell lines and bacteria, and cleaves DNA in a base-specific manner.[2] The attractive features of this molecule lie not only within the chemical structure being analogous to the neocarzinostatin chromophore,[3] itself a potent anticancer agent, but also in that it can invoke remarkably strong biological activities even without a stabilizing apoprotein carrier and a glycoside functionality that can accelerate the rate of DNA cleavage.[3e] In this regard, N1999-A2 serves as a leading enediyne-based antitumor agent with minimal functionality that is able to act on DNA selectively. We therefore focused on this unique, unstable, and stereochemically unknown compound and undertook the formidable challenge of devising an efficient strategy that would be flexible enough to ultimately construct a series of related highly strained systems.

Initially, we synthesized structure **1**, on the assumption that N1999-A2 has a stereochemistry that corresponds to the neocarzinostatin chromophore, and established that synthetic **1** was in fact a diastereomer of natural N1999-A2.[4] The physical and DNA cleavage data of **1** further suggested that the configuration of natural N1999-A2 was **2**. Herein we now wish to report on the realization of a concise and efficient strategy that has culminated in the first total synthesis of N1999-A2 (**2**) and to provide chemical evidence of a thiol-triggered aromatization of **2**. We also show for the first time the unique DNA cleavage profiles of a series of stereoisomers of N1999-A2 (**1**, **2**, *ent*-**1**, *ent*-**2**), which demonstrate that the stereochemical orientation of the C11-naphthoate unit plays a dominant role in defining efficiency and specificity in the DNA recognition and cleavage process.



Initial model investigations into the total synthesis of N1999-A2 were conducted according to our previous synthesis of **1**, all of which include a key cyclization step via C7−C8 bond formation using an enolizable aldehyde.[4] For instance, alcohol (**3**), which predominated over **7** after hydroboration−oxidation of the corresponding exo-olefin, was selected first and transformed to chloroacetate (**5**) in nine steps (Scheme 1).[4] In this case, however, the phenolic C2′-OTES ether cleaved more readily than the chloroacetate during any attempted chemoselective alcoholysis or hydrolysis step, and undesired products (**6**) always resulted.[5] Accordingly, epimer (**7**) was then used toward the total synthesis of **2** and converted to the unstable β,γ-unsaturated aldehyde (**8**) using established protocols (Scheme 1).[4] Contrary to our previous report on the synthesis of stereoisomer (**1**),[4] intramolecular acetylide addition of the enolizable aldehyde (**8**) proceeded rather poorly and gave the cyclized product (**9**) in only less than 10% yield. This unsatisfactory yield could not be improved and is consistent with that encountered in the synthetic study of the C-1027 chromophore, whereby the relative stereochemistry of the cyclization precursors as well as the bulkiness of protecting groups played a defining role for an efficient cyclization.[6a] In addition, like **4**, **9** displayed an undesired *cis*-relationship between C8−OH and C9−H, which also causes inefficiency in the formation of the C8−C9 double bond.[4]

With the information thus gained during this and parallel studies,[6] and for strategic reasons that will soon become clear, we remodeled our synthesis to cyclize at the C5−C6 bond in the key nine-membered precursor (**12**) (Scheme 2). This aldehyde (**12**) was rapidly accessed by coupling the readily available alkenyl iodide (**10**)[4] with the C2−C5 unit (**11**)[7] and performing an efficient protection and oxidation sequence. Application of the $LiN(TMS)_2$/$CeCl_3$-mediated cyclization protocol[8] to **12** at a relatively high temperature[4,6,9] efficiently afforded the highly strained nine-membered system (**13**) with the C4,C5-*trans*-diol stereochemistry, thereby poised for β-epoxide formation, as the major product (5:1) together with its *cis*-isomer[10] in a 68% combined yield. Besides giving higher yields than enolizable aldehyde systems, it should

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(2) Ando, T.; Ishii, M.; Kajiura, T.; Kameyama, T.; Miwa, K.; Sugiura, Y. *Tetrahedron Lett.* **1998**, *39*, 6495−6498.

(3) (a) Isolation of neocarzinostatin: Ishida, N.; Mizugaki, K.; Kumagai, K.; Rikimaru, M. *J. Antibiot.* **1965**, *18*, 68. (b) Chromophore structure: Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331−334. (c) Absolute configuration: Myers, A. G.; Proteau, P. J.; Handel, T. M. *J. Am. Chem. Soc.* **1988**, *110*, 7212−7214. (d) Total synthesis: Myers, A. G.; Hammond, M.; Wu, Y.; Xiang, J.-N.; Harrington, P. M.; Kuo, E. Y. *J. Am. Chem. Soc.* **1996**, *118*, 10006−10007; Myers, A. G.; Liang, J.; Hammond, M.;, Harrington, P. M.; Wu, Y.; Kuo, E. Y. *J. Am. Chem. Soc.* **1998**, *120*, 5319−5320. (e) Role of the aminoglycoside: Myers, A. G.; Kort, M. E.; Hammond, M. *J. Am Chem. Soc.* **1997**, *119*, 2965−2972; Myers, A. G.; Liang, J.; Hammond, M. *Tetrahedron Lett.* **1999**, *40*, 5129−5133. (f) *Neocarzinostatin, The Past, Present, and Future of an Anticancer Drug*; Maeda, H., Edo, K., Ishida, N., Eds.; Springer: Tokyo, 1997.

(4) Kobayashi, S.; Reddy, R. S.; Sugiura, Y.; Sasaki, D.; Miyagawa, N.; Hirama, M. *J. Am. Chem. Soc.* **2001**, *123*, 2887−2888.

(5) Presumably, this lack of chemoselectivity results from the naphthoate being located *cis* to the chloroacetate group and hindering ethoxide attack to this region. As a consequence, the free phenolic hydroxy group becomes unavoidably sulfonated during the next dehydration sequence (Tf_2O, 2,6-lutidine, DBU, −78 °C).

(6) (a) Sato, I.; Akahori, Y.; Iida, K.; Hirama, M. *Tetrahedron Lett.* **1996**, *37*, 5135−5138. (b) Sato, I.; Toyama, K.; Kikuchi, T.; Hirama, M. *Synlett* **1998**, 1308−1310.

(7) Enantiomerically pure **11** (mp 117−118 °C (hexane); $[\alpha]^{29.0}_D$ −17.2° (*c* 0.986, $CHCl_3$)) was synthesized from L-tartaric acid in eight steps: Fujita, K.; Nakai, H.; Kobayashi, S.; Inoue, K.; Nojima, S.; Ohno, M. *Tetrahedron Lett.* **1982**, *23*, 3507−3510; Nakatani, K.; Arai, K.; Hirayama, N.; Matsuda, F.; Terashima, S. *Tetrahedron* **1992**, *48*, 633−650.

(8) (a) For intramolecular acetylide addition to aldehydes to construct 10-membered enediynes, see: Kende, A. S.; Smith, C. A. *Tetrahedron Lett.* **1988**, *29*, 4217−4220; Cabal, M. P.; Coleman, R. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 3253−3255. (b) Effects of $CeCl_3$: Myers, A. G.; Harrington, P. M.; Kuo, E. Y. *J. Am. Chem. Soc.* **1991**, *113*, 694−695; Nishikawa, T.; Isobe, M.; Goto, T. *Synlett* **1991**, 393−395.

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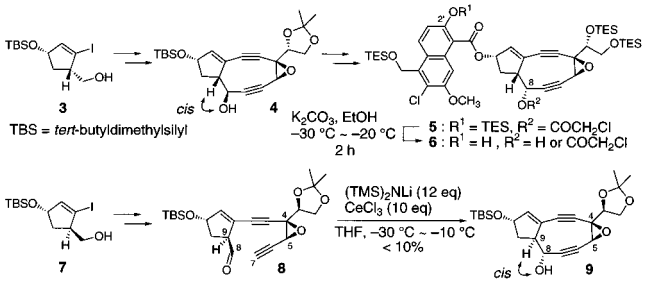
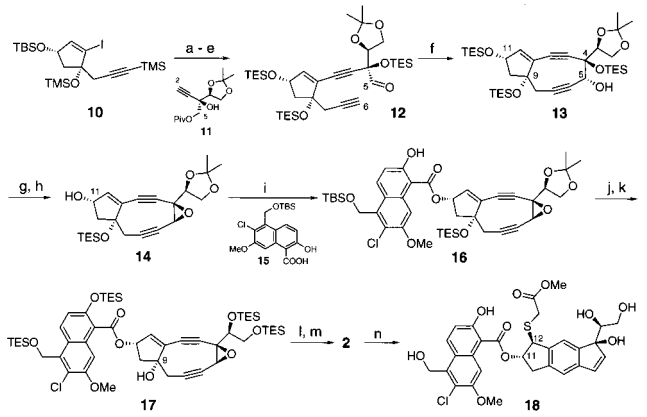
Scheme 1



Scheme 2[a]



[a] Reagents and conditions: (a) **11**, $(Ph_3P)_4Pd$, CuI, iPr_2NEt, DMF, rt, 2 h. (b) TBAF, THF, 0 °C to rt, 2 h, 91% (two steps). (c) TESOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 1 h. (d) DIBAL, CH_2Cl_2, −78 °C, 30 min, 87% (two steps). (e) $C_6H_4CO_2I(OAc)_3$, pyridine, CH_2Cl_2, rt, 2 h, 98%. (f) $(TMS)_2NLi$ (12 equiv), $CeCl_3$ (11 equiv), THF (3.8 mM), −30 °C to rt, 68%. (g) MsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C, 10 min. (h) TBAF (2 equiv), THF, −15 °C, 30 min, 63% (two steps). (i) **15**, DCC, THF, 0 °C, 1.5 h, 91%. (j) TFA−THF−H_2O (1:10:5), rt, 42 h, 60%. (k) TESOTf, 2,6-lutidine, CH_2Cl_2, −85 °C, 10 min, 63%. (l) Et_3N, DMAP, Ms_2O, CH_2Cl_2, 0 °C, 1.5 h. (m) TFA−THF−H_2O (1:10:5), 3 °C, 1.5 h, 58% (two steps). (n) methyl thioglycolate (0.5 M), Et_3N (0.5 M), 1,4-cyclohexadiene (1.0 M), THF (5 mM), 0 °C, 70 min, 23%.

be pointed out that $LiN(TMS)_2/CeCl_3$-mediated intramolecular acetylide addition reactions to aldehydes which possess neighboring tertiary alkyl silyl ethers always favor *trans*-diol systems.[6,9a]

Having realized an efficient and stereoselective cyclization to **13**, further work revealed that the C9−OH group must be protected during the next esterification step; otherwise, the desired mononaphthoate was produced only in low yields, even under selective 1,3-diol-monoesterification conditions involving Me_2SnCl_2.[11] Eventually, the alcohol (**13**) was mesylated, and most importantly, the C9-TES ether was left untouched at −15 °C with 2 equiv of TBAF during tandem formation of the β-C4,C5-epoxide and exposure of the C11-alcohol to give **14**. In this way, alcohol (**14**) could be cleanly esterified with naphthoic acid (**15**)[12] to give **16** in 91% yield.

On the basis of previous work, it was advisable at this stage in the synthesis to deprotect acetonide functionality and selectively reprotect alcohol functionality as TES ethers.[4] Therefore, the acetonide and silyl ethers of **16** were carefully hydrolyzed, without damaging the epoxide, using TFA−THF−H_2O (1:10:5) to give a pentaol, which was then selectively and strategically silylated to give the tetra-TES ether (**17**) with a free C9 alcohol. In the final and most critical reaction sequence, treatment of **17** with methanesulfonic anhydride and triethylamine in the presence of DMAP in CH_2Cl_2 at 0 °C afforded an unstable C8,C9-olefin, which was immediately purified by florisil column and treated with TFA−THF−H_2O (1:10:5) at 3 °C to produce the targeted product (**2**).[13] Synthetic N1999-A2 (**2**) was further purified by medium-pressure column chromatography (ULTRA PAK, DIOL 40A, ϕ11 × 300 mm, YAMAZEN Co. Ltd.) in 58% overall yield from **17**. The ^1H and ^{13}C NMR data, UV and CD spectra of synthetic N1999-A2 (**2**) are all identical to those of the natural product. Furthermore, substrate **2** in THF was shown to undergo cycloaromatization to adduct **18** at 0 °C upon addition of methyl thioglycolate, in the presence of a hydrogen donor and triethylamine,[3e] which suggests N1999-A2 would cleave DNA in a mechanism similar to that described for the neocarzinostatin chromophore.[1,3e,f,14]

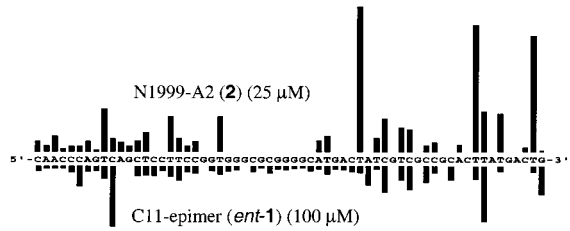


Figure 1. Histograms of DNA cleavage by synthetic N1999-A2 (**2**) (upper) and C11-epimer (*ent*-**1**) (lower). Incubations of 5′-^{32}P-labeled restriction fragment (SalI/NruI) from plasmid pBR322 were conducted with **2** (25 μM) and, separately, *ent*-**1** (100 μM) in the presence of dithiothreitol (40 mM) and calf thymus DNA (25 μg/mL) at 37 °C for 20 min and pH 7.0. The heights of the bars represent the relative cleavage intensities at the indicated bases.

It is noteworthy that in the defined stereochemistry of N1999-A2 (**2**) both the C4,C5-epoxide and C13-alcohol are antipodal to those of the neocarzinostatin chromophore, although **2**, **1**, and the neocarzinostatin chromophore all show similar base-specific DNA-cleavage profiles.[2,4] Therefore, we assumed that DNA cleavage efficiencies are mostly dependent on the C11-stereochemistry but not on the epoxide and C13-alcohol components. To verify this hypothesis the N1999-A2 stereoisomers (**1**, *ent*-**1**, *ent*-**2**) were synthesized by simple adaptation of the strategy described above and were then subjected to DNA-cleavage experiments.[15] Of special interest is, first, the fact that the DNA-cutting ability of the C11-epimer (*ent*-**1**) and the N1999-A2-enantiomer (*ent*-**2**) decreased dramatically as compared to that of N1999-A2 (**2**) and its diastereomer (**1**), both of which cleave DNA quite efficiently in a similar base-specific manner (autoradiograms are shown in Supporting Information). Histograms of DNA-cutting sites by N1999-A2 (**2**) and C11-epimer (*ent*-**1**) are depicted in Figure 1. Second, in the case of N1999-A2 (**2**) at 25 μM, single-strand breaks were observed mainly at thymidylic residues. Especially at these positions, the most strongly cleaved sites by the C11-epimer (*ent*-**1**) were shifted by one base-pair toward the 3′-teminal, although *ent*-**1** displayed a more random profile at 100 μM.

It is now clear that the (*S*)-configured C11-naphthoate unit plays a dominant role in not only governing the *trans*-orientation of thiol attack, which triggers aromatization, but also in the efficient recognition and base-specific cleavage of DNA. This is most thought-provoking regarding how the naphthoate unit intercalates and directs its reactive enediyne-core within DNA, and detailed work to decipher exactly how N1999-A2 binds and cleaves DNA is currently under scrutiny.

Supporting Information Available: Spectroscopic data for all new synthetic products, as well as reproductions of CD spectra of synthetic **2** and natural N1999-A2, ESI-TOF MS spectra of **2** and **18**, and electrophoresis autoradiograms of **1**, **2**, *ent*-**1**, and *ent*-**2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) In the case of the *cis*-isomer of **13**, the TES group at the C4-hydroxy position migrated to the newly formed secondary alcohol.

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(15) Detailed studies to synthesize N1999-A2 isomers will be disclosed in a full account.