Investigation of the Total Synthesis of N1999-A2: Implication of Stereochemistry

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N1999-A2 (**1**) is a novel member of the highly strained nine-membered enediyne antibiotic family. It is isolated from the broth filtrate of *Streptomyces* sp. AJ9493 and exhibits remarkably potent antitumor activity against various tumor cell lines.[1,2] It has a structure closely related to the neocarzinostatin chromophore (**2**) and interestingly displays DNA-cleaving selectivity similar to that of **2**,[3] but it lacks both the amino glycoside residue and an apoprotein unit to act as a stabilizing carrier. Therefore, N1999-A2 is expected to advance research into the roles of the naphthoate and core moieties in the binding and cleavage of DNA. However, the stereochemical definition of **1** remains to be determined. The highly strained, unstable, and densely functionalized structures of **1** and **2** represent a formidable synthetic challenge. In fact, only one successful total synthesis of **2** has yet been achieved by Myers despite numerous synthetic endeavors on this family.[3d,f,4] As part of our study into N1999-A2, we began with the synthesis of structure **3** on the assumption that the configuration of N1999-A2 corresponds to that of **2**. We report herein a total synthesis of **3**, which in fact proves not to be identical to natural N1999-A2, and formulate the configuration of natural N1999-A2.

Alcohol (**4**) was targeted as a precursor to **3**. We planned to synthesize **4** by unifying the cyclopentene moiety (**5**), the epoxydiyne unit (**6**)[4b] and the naphthoic acid (**7**).[5] The cyclopentene derivative (**5**) was prepared from enantiomerically pure **8**[6] (Scheme 1). Olefination of the ketone (**8**) with Tebbe reagent[7] followed by hydroboration yielded a 1:3 ratio of alcohol (**5**) and its C9-epimer, respectively.[8] The alcohol (**5**) was coupled with the epoxydiyne (**6**)[4b] using $(CH_3CN)_2PdCl_2$ as a catalyst under Sonogashira conditions[9] to afford **9** without interference from the dialkynyl epoxide functionality.[9b] The triethylsilyl group on the alkynyl terminal of **9** was selectively removed to give **10**. Dess−Martin oxidation[10] of **10** gave an unstable β,γ-unsaturated aldehyde (**11**), which was subjected without purification to $LiN(TMS)_2/CeCl_3$-mediated[11] cyclization[12] at a relatively high temperature.[13] Indeed, the enolizable aldehyde **11** was expected to cause problems in the cyclization step. However, the reaction reproducibly produced an alcohol (**12**) in a highly stereoselective manner. This is the first example of a successful intramolecular acetylide cyclization to an enolizable aldehyde.[3d,f,4,12,13] The alcohol (**12**) displayed a *syn*-relationship between C8−OH and C9−H as indicated by the coupling constant, $J_{H8,H9} = 10.0$ Hz and NOE experiments. However, all attempts to achieve *syn*-dehydration of **12** to obtain the corresponding C8,C9-olefin were unsuccessful.

To overcome this problem the C8-stereochemistry was inverted using the Mitsunobu protocol with chloroacetic acid[14] (Scheme 2). Careful treatment of the resulting chloroacetate (**13**) ($J_{H8,H9} = 3.0$ Hz) with TFA−THF−H_2O (1:10:5) at 0 °C liberated an alcohol (**14**), and then the naphthalene unit (**7**)[5] was attached to give the ester (**15**). After considerable investigation, we found

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(8) The stereochemistry was assigned by NOE experiments. The major epimer was also used for the synthetic study of **3**. However, we met serious difficulties on the way. For instance, during the chemoselective alcoholysis step that corresponds to **17**→**4**, the monochloroacetate was located *syn* to the naphthoate, and contrary to **17**, phenolic TES ether was always cleaved more readily.

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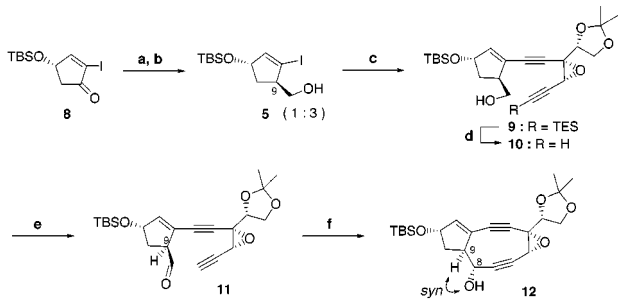
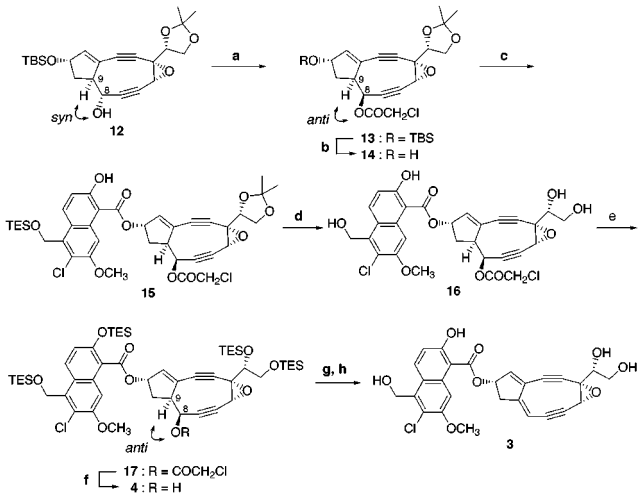
Scheme 1[a]



[a] Reagents and conditions: (a) $Cp_2TiCH_2AlClMe_2$, THF, rt, 79%. (b) 9-BBN, H_2O_2, NaOH, THF, rt, 88%. (c) **6**, $(CH_3CN)_2PdCl_2$, CuI, *i*Pr_2NEt, DMF, rt, 77%. (d) TBAF, THF, −78 °C, 82%. (e) $C_6H_4CO_2I(OAc)_3$, CH_2Cl_2, rt. (f) $(TMS)_2NLi$ (15 equiv), $CeCl_3$ (14 equiv), THF, −30 °C, 23% (two steps). TBS = *tert*-butyldimethylsilyl, 9-BBN = 9-borabicyclo-[3.3.1]nonane, TMS = trimethylsilyl, TBAF = tetrabutylammonium fluoride

Scheme 2[a]



[a] Reagents and conditions: (a) PPh_3, DEAD, $ClCH_2CO_2H$, toluene, −78 °C to rt, 53%. (b) TFA−THF−H_2O (1:10:5), 0 °C, 99%. (c) **7**, EDC·HCl, DMAP, CH_2Cl_2, rt, 69%. (d) TfOH, CF_3CH_2OH, 0 °C, 84%. (e) TESOTf, 2,6-lutidine, CH_2Cl_2, −78 °C. (f) K_2CO_3, EtOH, −25 °C to −5 °C, 30% (two steps). (g) 2,6-lutidine, Tf_2O, CH_2Cl_2, −78 °C; then DBU, −78 °C. (h) TFA−THF−H_2O (1:10:5), 0 °C, 30 min, 45% (two steps). TES = triethylsilyl, DEAD = diethyl azodicarboxylate, TFA = trifluoroacetic acid, EDC = 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, Tf = trifluoromethanesulfonyl, DBU = 1,8-diazabicyclo[5.4.0]-undec-7-ene.

that the acetonide group should be removed before forming the C8,C9-double bond. Therefore, **15** was hydrolyzed with the concomitant loss of the TES group using a catalytic amount of triflic acid in trifluoroethanol to give a tetraol (**16**). Global TES silylation of **16** and careful ethanolysis of the chloroacetate (**17**) below −5 °C to suppress cleavage of the phenolic TES ether afforded the desired alcohol (**4**). The use of a TBS group for protection of the tetraol **16** required deprotection conditions that were too harsh to allow for the survival of **3**. Treatment of **4** with Tf_2O and 2,6-lutidine in CH_2Cl_2 at −78 °C afforded a triflate that did not undergo spontaneous elimination at this low temperature. Accordingly, excess DBU was added after triflate formation was complete at −78 °C. The *anti*-E2-type dehydration

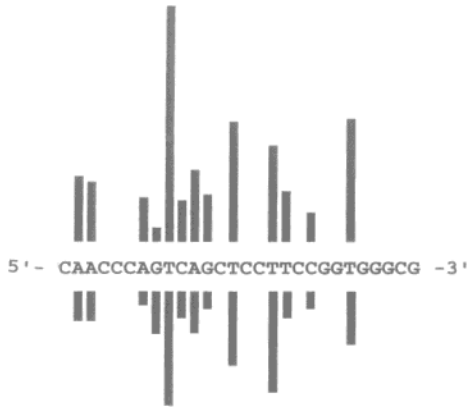
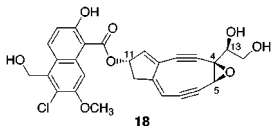


Figure 1. Histograms of DNA cleavage by synthetic **3** (upper) and natural N1999-A2 (lower). Incubations of 5′-^{32}P-labeled 323-base pair restriction fragment (SalI/NruI) from plasmid pBR322 were conducted with N1999-A2 (12.5−50 μM) and, separately, synthetic **3** (10−40 μM) in the presence of dithiothreitol (20 mM) and calf thymus DNA (5 mg/mL) at 37 °C and pH 7.0. The heights of the bars represent the relative cleavage intensities at the indicated bases.

proceeded rapidly to form the C8,C9-olefin, which was quickly purified by a short silica gel column. The resulting olefin was immediately treated with TFA−THF−H_2O (1:10:5) at 0 °C to produce the highly unstable epoxydienediyne (**3**).

Contrary to our initial assumption, the NMR data and HPLC profile[15] of synthetic **3** are not identical to those of the natural product, even though **3** was found to cleave DNA in a specific manner very similar to that of the natural product (Figure 1). The CD spectra of **3** and the natural product both showed a positive first Cotton effect [λ_{ext} 328 nm for natural; λ_{ext} 322 nm for **3** in DMSO-d_6/CD_3CN (1:1)], which is likely to be derived from the naphthoate chromophore being attached to C11 of identical configuration. The ^{1}H and ^{13}C NMR spectra of **3** and the natural product are listed in Supporting Information. The ^{1}H and ^{13}C chemical shifts of C13, C14, and C5 are essentially identical, which suggests that the relative configurations of the C4,C5-epoxide and the C13-alcohol are identical in the synthetic and natural product. Accordingly, we propose structure **18** as the natural product, which is the prime target of our total synthesis program toward N1999-A2, although the possibility of other diastereomers cannot be completely ruled out.



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Supporting Information Available: Reproductions of ^{1}H and ^{13}C NMR spectra and spectroscopic data for all new synthetic intermediates, and reproductions of ^{1}H and ^{13}C NMR, HPLC profile, UV, and CD spectra of **3** and natural N1999-A2 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Performed using an ODS column (Water Novapak C18, 8 × 100 mm) using 45% aqueous acetonitirile (1.2 mL/min) with UV detection (240 nm); retention times with co-injection: 10.0 min for the natural product, 9.5 min for **3**.