Pergamon

Tetrahedron Letters, Vol. 35, No. 14, pp. 2207-2210, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(94)E0299-D

Synthesis, Chemical Property, and Cytotoxicity of the Carzinophilin Congeners Carrying a 2-(1-Acylamino-1-alkoxycarbonyl)methylidene-1-azabicyclo[3.1.0]hexane System

Masaru Hashimoto, Miyoko Matsumoto, Kaoru Yamada, and Shiro Terashima*

Sagami Chemical Research Center, Nishi-Ohnuma, Sagamihara, Kanagawa 229, Japan

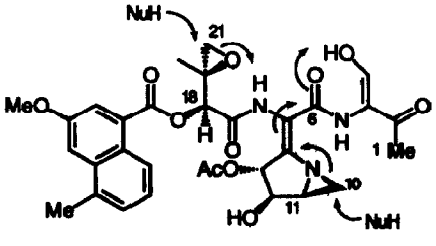
Key words: carzinophilin congeners, synthesis, chemical property, reaction with nucleophiles, cytotoxicity,

Abstract: *Synthesis of the title compounds was achieved by employing condensation of the 2-methoxy-1-pyrroline with ethyl nitroacetate and construction of 1-azabicyclo[3.1.0]hexane systems from 5-mesyloxymethylpyrrolidines as key steps. Some of these congeners which carry a C_6-C_{11} unit involving the naphthalene part, were found to exhibit prominent cytotoxicity and effectively alkylate nucleophiles at both the aziridine and epoxide moieties.*

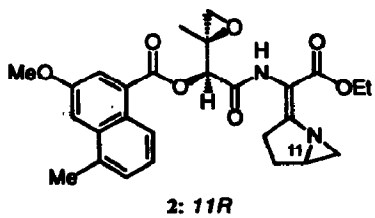
Carzinophilin (**1**) is an antitumor antibiotic isolated from *Streptomyces sahachiroi* by Hata *et al.* in 1954.[1] While the structure of **1** has been revised several times over 30 years,[2] Armstrong *et al.* reported in 1991 that ^{1}H- and ^{13}C-NMR spectra of **1** were superimposable on those of azinomycin B.[3] The latter compound is also an antitumor antibiotic having a characteristic 2-methylidene-1-azabicyclo[3.1.0]hexane system.[4] Since **1** is disclosed to be one of the strand cross-linking compounds for DNA,[5] it is anticipated that, as shown below, **1** could alkylate two molecules of nucleophiles at both the aziridine and epoxide moieties (the C_{10} and C_{11} positions) expressing its prominent cytotoxicity. These unique history, novel structure, and excellent cytotoxicity delineated above make **1** and its related compounds as attractive targets for total synthesis.[3,6]

We wish to report here a novel synthesis of the carzinophilin congeners (**2**, **3**, **4**, and *ent*-**4**) carrying a characteristic 2-(1-acylamino-1-alkoxycarbonyl)methylidene-1-azabicyclo[3.1.0]hexane system. The explored synthetic route features condensation of the 2-methoxy-1-pyrroline with ethyl nitroacetate and construction of 1-azabicyclo[3.1.0]hexane systems from 5-mesyloxymethylpyrrolidines as key steps. It was also disclosed that some of these congeners (**2** and **3**) which carry a C_6-C_{21} unit involving the naphthalene part, exhibit prominent cytotoxicity against P388 murine leukemia and effectively alkylate nucleophiles at both the aziridine and epoxide moieties.

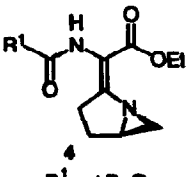
First, development of a novel synthetic route to **4** and *ent*-**4** was studied using (*R*)- and (*S*)-pyroglutamic acid as chiral templates. For convenience, only the synthetic scheme to **4** is shown in **Scheme 1**. Thus, the 2-pyrrolidone (**5**) readily accessible from (*R*)-pyroglutamic acid according to the reported procedure,[6i] was



carzinophilin (**1**) = azinomycin B



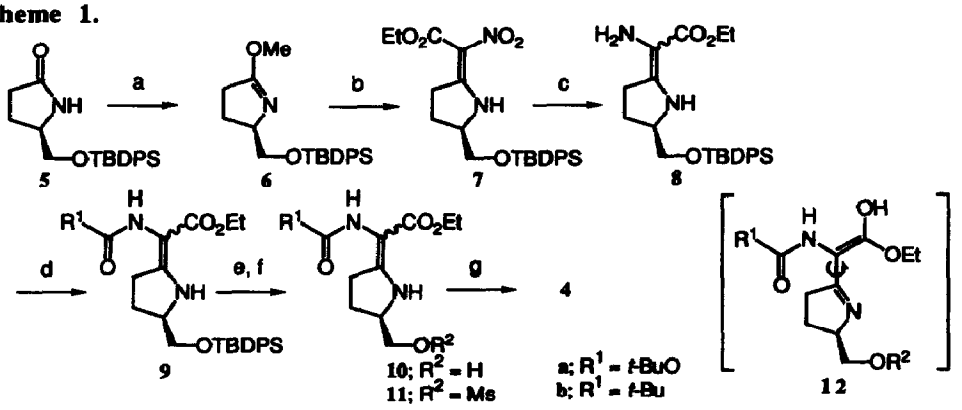
2: *11R*
3: *11S*



4
a: R^1 = *t*-BuO
b: R^1 = *t*-Bu

derived to methyl imidate (**6**).[7] After basic work-up, **6** was directly treated with an excess amount of ethyl nitroacetate to effect the condensation reaction, giving nitromethylidene (**7**).[8] Although **7** was obtained as a tautomeric mixture concerning the olefinic bond and the ratio of tautomers varied with solvents [1:1 (C_6D_6), 6:4 ($CDCl_3$), and 1:0 (CD_3OD)], the X-ray diffraction analysis obviously uncovered that **7** exists as a (*Z*)-isomer in a crystalline state. The nitro group of **7** was selectively reduced to provide unstable enamine (**8**), which was immediately acylated to afford 2-(1-acylamino-1-ethoxycarbonyl)methylidenepyrrolidines (**9a,b**) as mixtures of tautomers [(*E*):(*Z*) = both 7:1].[9] The tautomerization of **9** might occur through the rotation of enamine (**12**). After removal of the TBDPS group, the primary alcohols (**10a,b**) produced were converted to mesylate (**11a**) [(*E*)-isomer only] and a tautomeric mixture of mesylate (**11b**) [(*E*) : (*Z*) = 7 : 1], respectively. It was found that treatments of **9a,b** with KH in THF at room temperature smoothly underwent construction of the aziridine rings, furnishing **4a,b** in good yields. Since **4a,b** were very unstable against silica gel or Florisil, considerable amounts of the products decomposed during purification. For example, while **4a** was obtained in 56% yield after chromatography, the ^{1}H-NMR spectrum of the crude product obviously suggested the formation of **4a** in more than 80% yield. Starting with *ent*-**5** obtainable from (*S*)-pyroglutamic acid, *ent*-**4a,b** were similarly prepared by way of *ent*-**8**.

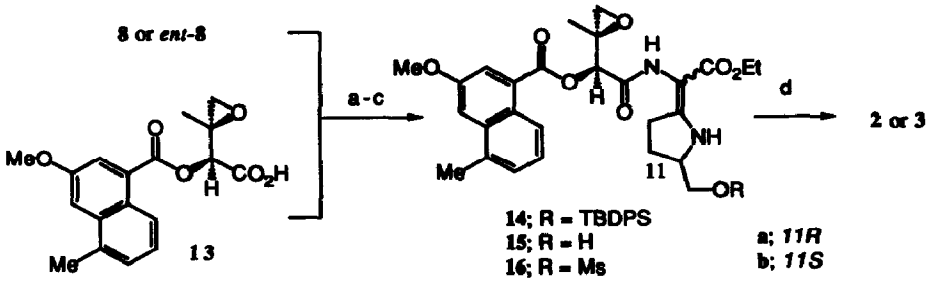
Scheme 1.



a) $(MeO)_2SO_2$, benzene, 60°C, 87% b) $O_2NCH_2CO_2Et$, 60°C, 67% c) H_2 (5 atm), 10%-Pd/C, toluene d) Boc_2O, AcOEt, 78% (**9a** from **7**) or PivCl, $NaHCO_3$, AcOEt, 84% (**9b** from **7**) e) TBAF, THF, 98% (**10a**), 95% (**10b**) f) MsCl, Et_3N, CH_2Cl_2, -78°C, 91% (**11a**), 74% (**11b**) g) KH, THF 56% (**4a**), 54% (**4b**)

With completion of a synthetic route to the 2-(1-acylamino-1-alkoxycarrbonyl)methylidene-1-azabicyclo-[3.1.0]hexane system, the preparation of **2** and **3** was next attempted. Thus, optically active epoxy acid (**13**) (>98%*ee*) which bears a C_6-C_{21} unit involving the naphthalene part, was prepared according to the protocols explored by Shibuya *et al.*[6a] and Hirama *et al.*[6f] with slight modifications. Condensation of **8** *in situ* produced from **7**, with **13** was effected by means of DCC-HOBT method, affording **14a** as a tautomeric mixture. This was elaborated to **16a** by a similar reaction sequence to that described above. In this case, the aziridine moiety could be constructed more cleanly with KHMDS than with KH, furnishing **2**.[10] The crude yield of **2** could not be estimated by its ^{1}H-NMR spectrum due to spectral crowding . Although **2** was found to be very unstable similarly to **4a,b**, short preparative TLC performed within 10 min made it possible to isolate **2** almost in a pure state. In a similar fashion, **3** was prepared by employing *ent*-**8** *in situ* prepared from *ent*-**7**. Being different from their synthetic intermediates (**7-11** and **14-16**), all the carzinophilin congeners (**2**, **3**, **4**, and *ent*-**4**)

Scheme 2.

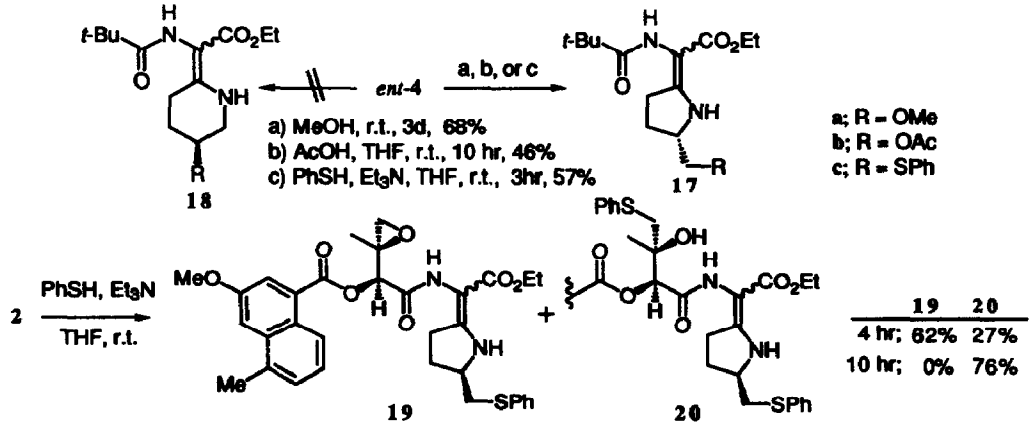


a) DCC, HOBT, THF, 51% (14a), 62% (14b) b) TBAF, THF, 76% (15a), 68% (15b) c) MsCl, Et_3N, -78°C, 88% (16a), 92% (16b) d) KHMDS, THF, 34% (2), 21% (3)

produced here were found to consist of single stereoisomers. Stereochemistries of the olefinic moieties in these compounds were tentatively assigned as (*E*)-configurations by their ^{1}H-NMR spectra.[11]

With various carzinophilin congeners in hand, their chemical property was next examined using *ent*-4.[6i] A pure sample of *ent*-4 was found to be stable in $CDCl_3$ at 0 °C for a week. However, treatment with AcOH or PhSH/Et_3N brought about selective cleavage of the aziridine ring at the C_{10} position (carzinophilin numbering) to yield the pyrrolidine (**17**). No formation of the piperidine (**18**) was detected under these conditions. The same opening of the aziridine ring was effected even by treating with MeOH. In the case of more functionalized **2**, two molecules of PhSH were found to attack successively at the C_{10} and C_{21} positions, producing bisadduct (**20**). Since the reaction performed in a short time afforded monoadduct (**19**) as a major product, **20** is anticipated to be derived by way of **19**. These observations might mimic the anticipated double alkylation ability of **1** for DNA.

Scheme 3.



Finally, *in vitro* cytotoxicity assay against P388 murine leukemia was examined using **2**, **3**, **4**, and *ent*-**4** as well as **19** and **20**. It was found that highly functionalized **2** and **3** exhibit prominent cytotoxicities [IC_{50} (μg/mL): 0.0023 (**2**), 0.0036 (**3**)] which are almost equal to that of adriamycin [IC_{50} (μg/mL): 0.0020] widely employed for clinical uses. It is also worth noting that the compounds lacking the epoxide moiety (**4** and *ent*-**4**), the aziridine part (**19**), or both (**20**), showed the cytotoxicity obviously weaker than those carrying both the functionalities [IC_{50} (μg/mL): >10 (**4a**), 9.6 (*ent*-**4a**), 9.3 (**4b**), 8.7 (*ent*-**4b**), 0.035 (**19**), and 1.9 (**20**)].[12]

Based on the results accumulated in these studies, the total synthesis of **1** is in progress in our laboratory.

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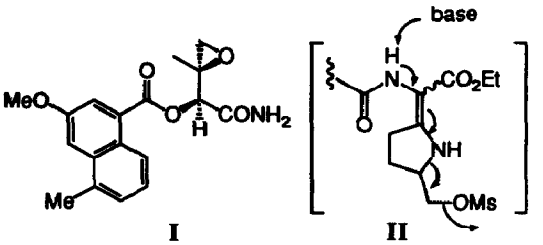
9) In the ^{1}H-NMR spectra measured in $CDCl_3$, the signals due to the allylic protons of major (*E*)-tautomers of **8** and **9** appeared at lower fields (3.05-3.35 ppm) than those of minor (*Z*)-tautomers (2.70-2.90 ppm). Célérier, J. P.; D-Marchalant, E.; Lhommet G. *J. Heterocycl. Chem.*, **1984**, *21*, 1633.
10) In both the aziridine formation from **16a** and the aziridine cleavage of **2** with PhSH, the formation of a small amount of amide (**I**) was always observed. I was identified by spectral comparisons with the natural product isolated by Yokoi *et al.*[4b] along with azinomycins and with an authentic sample independently synthesized by Shibuya *et al.*[6c] Formation of **I** in the former reaction might be explained by the attack of a base on the other amide proton, resulting in elimination of the mesyl group as shown in **II** and subsequent hydrolysis of the produced imine. In the latter reaction, **I** might be produced by simple hydrolysis of the enamine part. These results suggest that natural **I** might be derived from **1** *in vivo*. Contrary to the reported results,[4b] **I** was found to show strong *in vitro* cytotoxicity against P388 murine leukemia [IC_{50} (μg/mL): 0.0036]. We thank Prof. Masayuki Shibuya (University of Tokushima) for kindly providing us with an authentic sample and spectral data of **I**.



I **II**

11) The signals of methylene protons in the ethyl groups of **2**, **3**, **4**, and *ent*-**4** appeared at lower fields than those of the corresponding major (*E*)-tautomer of synthetic intermediates (**9**-**11** and **14**-**16**). This might be construed by the locations of the ethyl groups of **2**, **3**, **4**, and *ent*-**4** not only *syn* to but also coplanar to their aziridine rings. Although Armstrong *et al.*[6h,j] and Coleman *et al.*[6k] determined the stereochemistries of their model compounds by using NOE technique, no NOE useful for assigning the stereochemistries of our bicyclic compounds was observed in our case. Incidentally, NOEs between the amide proton and the protons on the pyrrolidine ring were not described by Yokoi *et al.* in their report concerning the structure determination of azinomycins.[4b]
12) In addition to **2**, **3**, **4**, and *ent*-**4**, various model compounds related to **1** were prepared in a similar manner to that reported. Results of their *in vitro* cytotoxicity assay will be reported in due course.

(*Received in Japan* 24 *December* 1993)