[17] a) A. H. Fainberg, S. J. Winstein, *J. Am. Chem. Soc.* **1956**, *78*, 2767–2774; b) J. Mattay, *Synthesis* **1989**, 233–252.

[18] A non-chain radical mechanism that involves C16–Br bond homolysis and intramolecular addition of the resultant aryl radical to the indole nucleus cannot, at this point, be ruled out.

[19] We are grateful to Professor William Fenical (Scripps Institute of Oceanography) for a generous gift of natural (−)-diazonamide A.

[20] Synthetic **1a** appears (^{1}H NMR) as an ≈4:1 mixture of C11 epimers. However, unpredictable degradation prevents detailed characterization of these materials. Protected derivative **27** is serviceable with respect to handling and analysis.

[21] Diketopiperazine formation appears to be a competing pathway when a C37 Fmoc-amine-protected congener of **27** is treated with DBU in THF. Fmoc = fluorenylmethyloxycarbonyl.

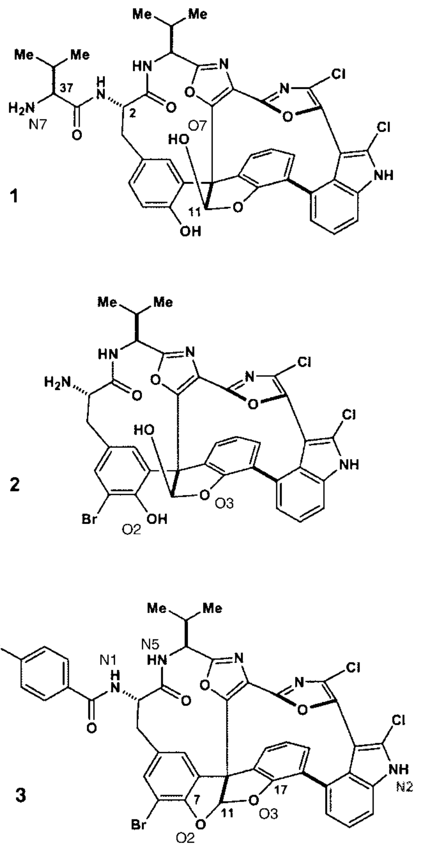
[22] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-174087. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Total Synthesis of Nominal Diazonamides—Part 2: On the True Structure and Origin of Natural Isolates[**]

Jing Li, Anthony W. G. Burgett, Lothar Esser, Carlos Amezcua, and Patrick G. Harran*

In the preceding communication we described a fully synthetic pathway to the structure proposed for (−)-diazonamide A (**1**, Scheme 1).[1] This material is not identical to the natural product, which raises the obvious question: What is the true structure of diazonamide A? Herein we provide an answer. In addition, we report that defined synthetic entity **8** induces, with equal potency, a toxic phenotype in cell culture indistinguishable from that produced by natural (−)-diazonamide A.

Spectroscopic data for the heterocyclic cores of diazonamides A and B are nearly identical.[2] So when the crystal structure of a *p*-bromobenzamide derivative of diazonamide B was reported as **3**,[2a] the diazonamide A assignment seemed only to require reconciling its exact mass (765.1998 amu)[3] within the same framework. The molecular formula $C_{40}H_{35}N_6O_6Cl_2$ is consistent with this mass (Δ −0.3 ppm) although it was thought to reflect a desiccated form of the molecule as a result of a C11 hemiacetal that loses water during HRMS analysis. Likewise, the C11 diphenylacetal reported in **3** was thought a result of net dehydration occurring during derivatization of diazonamide B with *p*-bromobenzoyl chloride. Hemiacetal functionality was considered a necessary part of both natural products to accommodate a small vicinal coupling between C11H and an exchangeable, one-proton resonance just over $\delta = 7$ in ^{1}H NMR spectra ([D$_6$]DMSO). The diazonamide A assignment (**1**) was then completed by incorporation of a terminal valine residue which emanates from the C2 amine. In the context of a C11 hemiacetal, this designation does coincide with the high resolution mass measurement but, unfortunately, with little else.



Scheme 1. Initial diazonamide structure assignments.

Acid digests of diazonamide A do not produce valine.[2b] ^{1}H NMR (360 MHz, [D$_6$]DMSO) indicates the presence of two isopropyl groups in the molecule, but the N7 protons are reported as a sharp, one-proton doublet at $\delta = 5.46$. This resonance is, in turn, coupled (5.9 Hz) to the C37 methine hydrogen at $\delta = 3.75$.[4] In a triacetate derivative, the C37 proton shifts downfield to $\delta = 5.11$, although it now appears as a doublet rather than the more complex pattern one might expect for a C37 acetamide.[5] Moreover, in non-acetylated material, C37 resonates at $\delta = 76.9$ (50 MHz, [D$_4$]MeOH)—considerably downfield from the corresponding carbon atom

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in a typical valine free-base.[6] We believe these observations are consistent with the C37 substituent in natural diazonamide A being an alcohol rather than an amine.[7]

For this to be true, the NH_2 to OH change dictates that a compensatory permutation be made at another position in the structure to rectify the attendant increase by 1 Da in molecular mass.[8] This requires revising the X-ray structure assigned as **3**. Notably, the exact mass of diazonamide B is 743.0340 amu.[2] However, the structure proposed for this material (**2**) has the formula $C_{35}H_{26}N_5O_6Cl_2Br$ and an $[[M^+{+}H] - H_2O]$ ion has the calculated mass 744.0416 amu. The formula $C_{35}H_{25}N_6O_4Cl_2Br$ $[[M^+{+}H] = 743.0576$ amu] is more consistent with the observed mass ($\Delta = 2.4$ ppm) and this suggests that a protonated nitrogen atom in diazonamide B has been mistaken for oxygen in **3**.

C11 hemiacetals in natural diazonamides are not indicated by mass spectrometry. Moreover, synthetic materials with this functional group (namely, **1**)[1] ionize intact, which makes the O2 or O3 assignment suspect. In the structure assigned as **3**, the observed C7–O2 bond length (1.371 Å) falls within the range typical for aryl C–O bond distances (1.353–1.409 Å) and deviates by just 1.5 σ (σ = standard deviation) from the mean value of 1.385 Å (based upon 36 bonds in 20 related substructures found within the Cambridge Crystallographic Database).[9] However, the C17–O3 bond, likewise expected to be an aryl C–O bond, is measured at 1.433(16) Å. This is 0.048 Å (3 σ) longer than the mean and, notably, exceeds the maximal value (1.409 Å) observed for a bond of this type. Atom O3 also displays an unusually large thermal motion for an atom in a rigid group (Figure 1). The average B-factor (B_{eq}) in the core (O3 excluded) is 4.8(3) $Å^2$ while the temperature factor of O3 itself is 7.42 $Å^2$—or 8.7 σ above the average.[10] This indicates that the O3 assignment should be changed to an element with fewer electrons and a larger covalent radius.

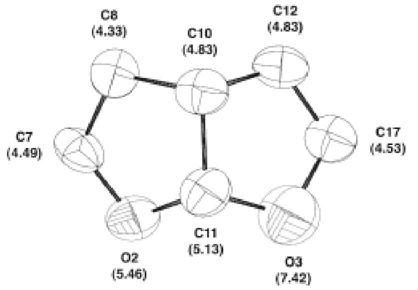
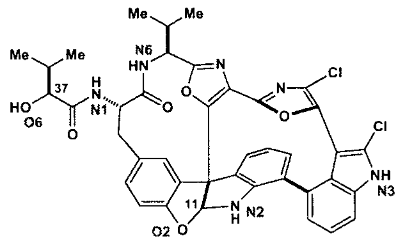
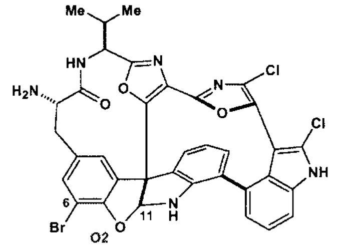


Figure 1. Partial reconstruction (ORTEP; 50 % probability thermal ellipsoids) of the X-ray structure refinement assigned as **3** (CCDC ref. code = JIMBUC). Numbers in parentheses are equivalent isotropic displacement coefficients (B_{eq}) in units of $Å^2$.

When taken together, these data are consistent with the electron density assigned as O3 being a protonated nitrogen atom and, by extension, the actual structure of (−)-diazonamide B being C11 diarylaminal **5** (Scheme 2). This change, in combination with an *S*-configured C37 alcohol,[11] gives **4** as our revised structure of (−)-diazonamide A. We have performed an $^1H/^{15}N$-HSQC experiment on natural diazonamide A that, when interpreted in light of extensive $^1H/^1H$ and $^1H/^{13}C$ correlations obtained for the compound,[2b] clearly



4 (−)-diazonamide A (revised Structure)



5 (−)-diazonamide B (revised Structure)

Scheme 2. Revised structures of (−)-diazonamides A and B.

supports this assignment. The $^1H/^{15}N$-HSQC experiment[12] allows protons attached to nitrogen to be uniquely identified. The two-dimensional spectrum shown in Figure 2[13] indicates four such connectivities in the natural product: $\delta = 12.82$ (N3H); $\delta = 8.66$ (N6H); $\delta = 7.68$ (N1H); $\delta = 7.16$ (N2H). The proton resonance at $\delta = 7.16$ is coupled to C11H (DQF-COSY) and was originally assigned as O7H in **1**. Moreover, the exchangeable one-proton doublet at $\delta = 5.46$, first identified as $N7H_2$, is not coupled to ^{15}N—consistent with our C37 hydroxy model.

To demonstrate these issues further, we have synthesized L-valine and (*S*)-α-hydroxy isovaleric acid conjugates of our synthetic, C11 diphenyl acetal core structure **6**[1] (**7** and **8**, respectively; Scheme 3). Alcohol **8** is > 50-fold more potent than amine **7** at inhibiting the growth of human ovarian adenocarcinoma OVCAR-3 in vitro (Table 1). Within experimental error, **8** and natural diazonamide A are equipotent in this assay.[14] Compound **8** is also an antimitotic agent. Populations of OVCAR-3 accumulate as tetraploid (4N) when exposed to low doses of **8** (30 nM). Those cells remaining viable persist with two copies of their genome for the duration of the experiment.[15] The effect is similar to positive antimitotic controls (taxol and vinblastine) and indistinguishable from that produced by 30 nM diazonamide A treatment.

To look for effects on mitosis directly, we synchronize a monkey kidney epithelial cell line (BS-C-1) in S phase, treat individually with **8** and diazonamide A, and image the tubulin cytoskeleton by immunofluorescence microscopy 9 hours later. In contrast to a vehicle-alone control (Figure 3 A), a more significant fraction (ca. 30 %) of BS-C-1 cells treated with 100 nM **8** or diazonamide A appear mitotic. Moreover, virtually none of these cells are able to construct a normal bipolar mitotic spindle (Figure 3 B,C). The phenotype is pleotropic at the level of precise microtubule and chromosomal (data not shown) organization although, importantly, the

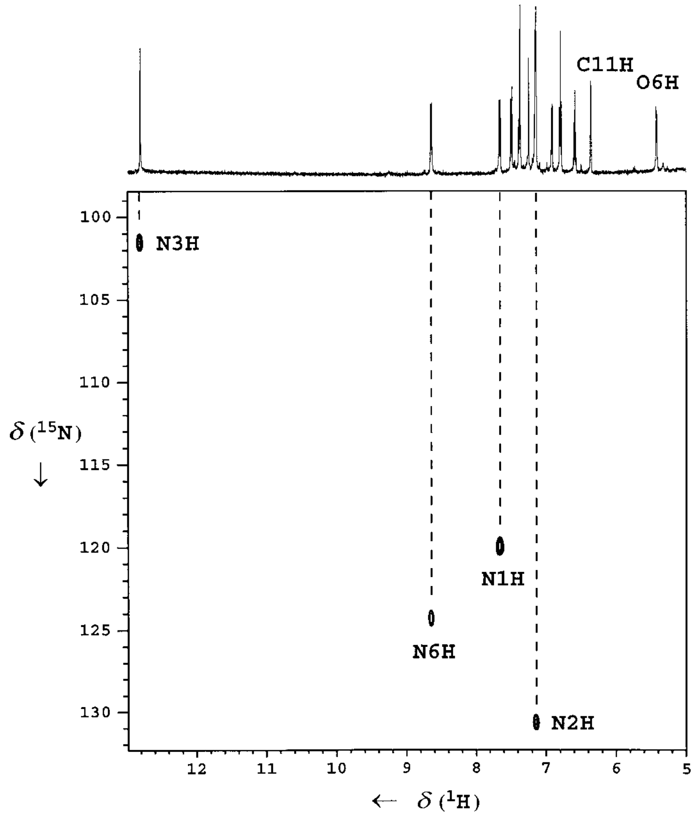
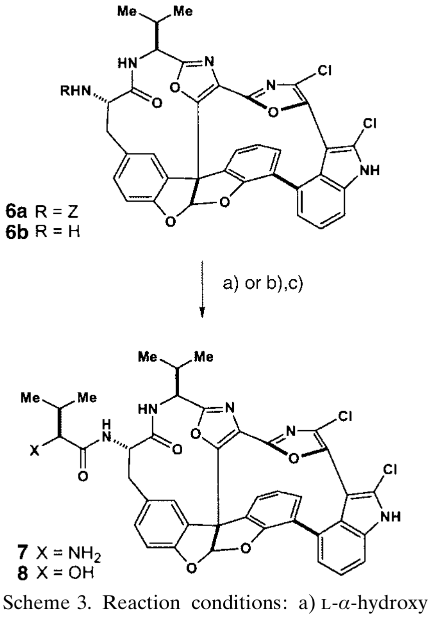


Figure 2. Partial $^{1}H/^{15}N$-HSQC spectrum (500 MHz, [D$_6$]DMSO) of natural diazonamide A.



Scheme 3. Reaction conditions: a) L-α-hydroxy isovaleric acid, $(EtO)_2P(O)CN$, N-methylmorpholine, THF (**8**; 95 %); b) Z-L-Val-OH, TBTU, iPr$_2$NEt, DMF; c) 10 % Pd/C, 1 atm H_2 (g), MeOH (**7**; 92 %, 2 steps). Z = benzyloxycarbonyl; TBTU = 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate.

Table 1. In vitro cytotoxicity assays.[a]

Compound	GI$_{50}$ [nM]
natural diazonamide A	8
8	16
8 (*epi*-C37)	191
7	845
6a	> 10000
3	> 10000
paclitaxel	8

[a] Growth inhibition determined for human adenocarcinoma OVCAR-3 after 48 h compound treatment with the CellTiter-Glo viability assay (Promega).

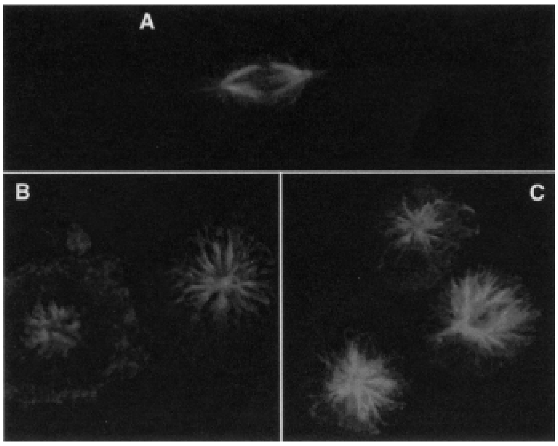
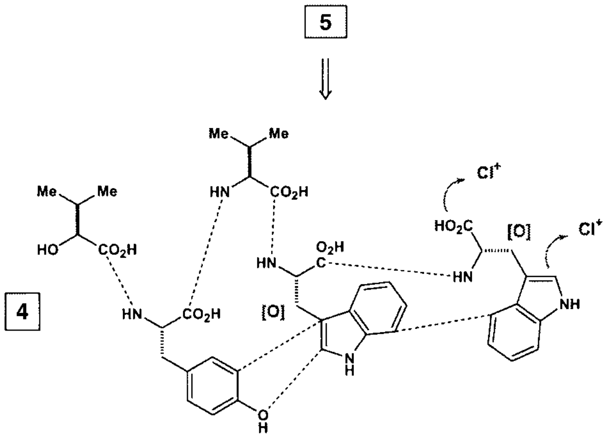


Figure 3. Mitotic BS-C-1 cells 9 hours post release from thymidine block. Confocal immunofluorescence microscopy showing tubulin-based assemblies formed in the presence of: **A**) 0.01 % EtOH; **B**) 100 nM **4** (natural diazonamide A), and **C**) 100 nM **8**.

range of effects is similar for both compounds. We are confident that, by these preliminary measures, acetal **8** is a functional equivalent of (−)-diazonamide A. This is a key discovery in that it validates the ability of our existing synthesis to fuel more sophisticated biochemical and molecular biological aspects of diazonamide research.

In closing, our structure revisions appear to clarify a biosynthetic lineage between diazonamide polycycles and four common amino acids (Scheme 4). Initial assignments (namely, **1** and **2**) seemed to require invoking an ambiguous hybrid assembly of amino acid and aromatic polyketide segments.[2] Details notwithstanding, the polyheterocyclic core now appears to be a derivative of an oxidized, 4,7-linked ditryptophan unit with the macrolactam ring being formed by



Scheme 4. A plausible biosynthetic origin of revised diazonamide structures.

a net oxidative cycloaddition between tyrosine and tryptophan. We are not aware of precedent for the latter event although the outcome is generally reminiscent of the production of dehydrodiconiferyl alcohols during lignan biosynthesis.[16]

Experimental Section

8: $R_f = 0.58$ (75% EtOAc/benzene); $[\alpha]_D^{25} = -154.8°$ ($c = 0.47$, MeOH); IR (film): $\tilde{\nu} = 3280$, 2965, 1659, 1652, 1645, 1520, 1490, 1441, 1053, 910, 753 cm^{-1}; ^{1}H NMR (400 MHz, [D$_4$]MeOH): $\delta = 7.51$ (d, $J = 2.0$ Hz, 1H), 7.47 (dd, $J = 1.2$, 8.0 Hz, 1H), 7.36 (app t, $J = 8.0$ Hz, 1H), 7.27 (dd, $J = 2.0$, 8.4 Hz, 1H), 7.20 (app dd, $J = 1.2$, 7.8 Hz, 2H), 7.07 (dd, $J = 1.2$, 7.6 Hz, 1H), 6.93 (app t, $J = 7.6$ Hz, 1H), 6.88 (d, $J = 8.4$ Hz, 1H), 6.84 (s, 1H), 4.98 (d, $J = 6.0$ Hz, 1H), 4.61 (dd, $J = 3.2$, 11.6 Hz, 1H), 3.89 (d, $J = 4.0$ Hz, 1H), 3.47 (app t, $J = 8.4$ Hz, 1H), 2.81 (dd, $J = 3.2$, 12.8 Hz, 1H), 2.34–2.26 (sym 6-line m, 1H), 2.14–2.06 (sym 10-line m, 1H), 1.10 (d, $J = 6.8$ Hz, 3H), 1.03 (d, $J = 6.8$ Hz, 3H), 0.96 (d, $J = 6.8$ Hz, 3H), 0.92 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR (75 MHz, [D$_4$]MeOH): $\delta = 175.8$, 175.2, 163.2, 159.8, 159.3, 154.9, 153.7, 141.8, 136.7, 132.4, 131.8, 131.3, 131.2, 130.7, 130.4, 129.5, 128.7, 127.9, 127.6, 127.0, 125.2, 124.2, 124.1, 124.1, 122.7, 119.7, 112.5, 111.8, 98.1, 77.0, 62.2, 57.3, 56.5, 39.0, 33.4, 31.6, 19.7, 19.6, 18.7, 16.6; ES-MS: calcd for $C_{40}H_{33}Cl_2N_5O_7$ [M^++H]: 766.18, found: 766.30; calcd for $C_{40}H_{33}Cl_2N_5O_7$ [M^- − H]: 764.16, found: 764.31. HR-FAB-MS calcd for $C_{40}H_{33}Cl_2N_5O_7$ [M^++Li]: 772.1917, found: 772.1962.

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[1] J. Li, S. Jeong, L. Esser, P. G. Harran, *Angew. Chem.* **2001**, *113*, 4901–4906; *Angew. Chem. Int. Ed.* **2001**, *40*, 4765–4770.

[2] a) N. Lindquist, W. Fenical, G. D. Van Duyne, J. Clardy, *J. Am. Chem. Soc.* **1991**, *113*, 2303–2304; b) N. Lindquist, Ph.D. thesis, University of California San Diego (USA), **1989**.

[3] The high resolution FAB mass spectrum of diazonamide A shows a cluster of six ions between 765 and 770 amu, the relative intensity of which indicates the presence of two chlorine atoms. Heavy-atom analysis confirms that chlorine is the only halogen present.

[4] The corresponding C37 methine resonance in synthetic **1** appears at $\delta = 3.2$ (400 MHz, [D$_6$]DMSO) and is broadened.

[5] Peracetylated diazonamide A shows three methyl singlets at $\delta = 2.87$, 2.23, 2.16 in its ^{1}H NMR spectrum (360 MHz, CDCl$_3$) and two new IR absorbances at 1760 cm^{-1} and 1725 cm^{-1}.

[6] BioMagResBank is a searchable database of NMR spectroscopic data on proteins, peptides, and nucleic acids. See: http://www.bmrb.wisc.edu/index.html.

[7] This requires that diazonamide A be a conjugate of α-hydroxy isovaleric acid (HIV). The ^{1}H NMR spectrum (300 MHz, [D$_6$]DMSO) of HIV (L-form, Fluka) shows that Cα resonates at $\delta = 3.73$ (^{13}C NMR: $\delta = 74.5$) and is weakly coupled to the exchangeable carbinol proton at $\delta = 5.0$.

[8] We initially considered that misassignments were made only in the C2 amine side chain. An α-hydroxy amidine congener of **1** was therefore prepared. This material incorporates a C37 carbinol and does have the same net atomic composition as **1**. However, chromatographic and spectroscopic properties of the compound rule it out as a possibility.

[9] See supporting information.

[10] For comparison, we note that $B_{eq} = 6.00$ and 5.35 Å^2, respectively, for O2 and O3 in the X-ray structure refinement of synthetic diphenyl acetal **28**.[1]

[11] ^{1}H NMR spectra of **8** and its C37 epimer (derived from D-α-hydroxy isovaleric acid) are near identical. We assign C37-*S* stereochemistry in **4** based upon relative potencies in cell-based assays. See Table 1.

[12] L. E. Kay, P. Keifer, T. Saarinen, *J. Am. Chem. Soc.* **1992**, *114*, 10663–10665.

[13] ^{1}H/^{15}N-HSQC, ^{1}H/^{13}C-HSQC, and DQF-COSY experiments were recorded at 25°C on a 500 MHz Varian Inova spectrometer. Data was processed using NmrPipe and analyzed with NMRView.

[14] The trace amount of natural diazonamide A (ca. 700 μg) available for these experiments makes it likely that weighing error alone could approach a factor of two.

[15] The effect of compound treatment on cell ploidy was evaluated by fluorescence-activated cell sorting (at 4 h intervals over 16 h). Experimental results are provided as supplementary information.

[16] D. R. Gang, M. A. Costa, M. Fujita, A. T. Dinkova-Kostova, H. Wang, V. Burlat, W. Martin, S. Sarkanen, L. B. Davin, N. G. Lewis, *Chem. Biol.* **1999**, *6*, 143–151.