I) to provide the target dimer **7** as a stable yellow oil[11] in 8% overall yield.

DNA-binding of **7** was initially observed through thermal denaturation studies with calf thymus DNA[13] (ΔT_m >15.1 °C for a 5:1 ratio of DNAp:ligand at 37 °C for 18 h). Cross-linking efficiency was investigated using an agarose gel electrophoresis assay[14] based on the principle that, following complete denaturation of linear pBR322 DNA, an interstrand cross-link results in renaturation to the double-stranded form in a neutral gel. The results indicate that **7** is a remarkably efficient cross-linking agent (Figure 1). After 2 h at 37 °C, cross-linking is measurable down to at least 0.01 μM (drug:nucleotide ratio = 0.025), with >90% cross-linking at 0.4 μM (drug:nucleotide ratio = 1.0). In the same assay, it is 50-fold more effective than the major groove cross-linkers mechlorethamine and cisplatin,[14] approximately 300 times more efficient than melphalan,[14] and similar in efficiency to rigid CC-1065 dimers such as U77779.[5c] However, in contrast to the weaker-binding C7-linked PBD dimer,[6,15,16] no reversibility of cross-linking was observed after incubation for 16 h at 37 °C and pH 4-10. In addition, compound **7** is highly cytotoxic, and comparison with the monomer **8** (Table I) emphasizes the biological consequences of DNA cross-linking.

Extensive modeling studies of **7** with $d(CGYGXXCYCG)_2$ have suggested that spatial separation of the PBD units is optimal for spanning six base pairs with a preference for 5′-PuGATCPy or 5′-PyGATCPu sequences, and that it actively recognizes the embedded $d(GATC)_2$ sequence. The self-complementary 10-mer $d(CICGATCICG)_2$[17] was designed to investigate the interstrand cross-linking potential of **7**. ^{1}H-NMR examination of the 1:1 adduct showed that the duplex is cross-linked symmetrically via the minor groove N2 positions of the guanines, with 11(*S*),11′-(*S*)-stereochemistry in the ligand and minimal distortion of the helix. The model was subsequently refined at the all-atom level by an interproton NOE distance-restrained dynamic annealing procedure using X-PLOR 2.1[18] (Figure 2).

In summary, C8-linked PBD dimers of this class are highly efficient irreversible DNA cross-linking agents that may have potential application in cancer chemotherapy and as biochemical tools.

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Registry No. 1, 3018-48-2; **2**, 130107-96-9; **3**, 140658-44-2; **4**, 140658-45-3; **5**, 140658-46-4; **6**, 140658-47-5; **7**, 140676-21-7; **8**, 89824-22-6; $d(CICGATCICG)_2$, 140658-48-6; $I(CH_2)_3I$, 627-31-6; vanillic acid, 121-34-6; (2*S*)-pyrrolidine-2-carbaldehyde diethyl thioacetal, 105089-88-1.

(11) Compound **7** is stable on storage under dry conditions: $[\alpha]^{25}_D$ +330° (*c* = 0.6, $CHCl_3$). All new compounds were characterized by standard spectroscopic methods.

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Formation of 3-Amino-5-hydroxybenzoic Acid, the Precursor of mC_7N Units in Ansamycin Antibiotics, by a New Variant of the Shikimate Pathway†

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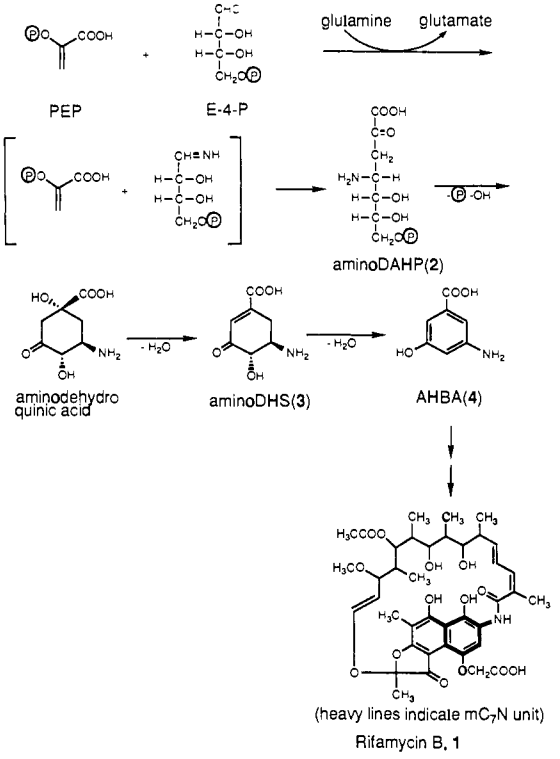
A variety of antibiotics, notably the ansamycins, e.g., rifamycin B[1] (**1**), and the mitomycins,[2] contain a biosynthetically unique moiety, called an mC_7N unit, consisting of a six-membered carbocyclic ring carrying an extra carbon and a nitrogen in a meta arrangement. Extensive tracer[2,3] and genetic[4] experiments have demonstrated the shikimate pathway origin of this mC_7N unit, although neither shikimic acid[3a,c,5,6] nor dehydroquinic acid[3d] were incorporated, and have identified 3-amino-5-hydroxybenzoic acid (AHBA, **4**) as its proximate precursor.[7] The nitrogen of the mC_7N unit is linked to the carbon corresponding to C-5, not C-3 of, for example, dehydroshikimic acid,[3d,8-11] and its origin, in the case of **1**, has been traced to the amide nitrogen of glutamine.[12] This and mechanistic considerations led us[10] to propose the pathway shown in Scheme I for the formation of **4**. The key

Scheme I



feature is the suggested operation of a modified DAHP synthase containing an additional protein subunit which binds and hy-

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† This paper is dedicated to Professor Helmut Simon, Technical University Munich, on the occasion of his 65th birthday.
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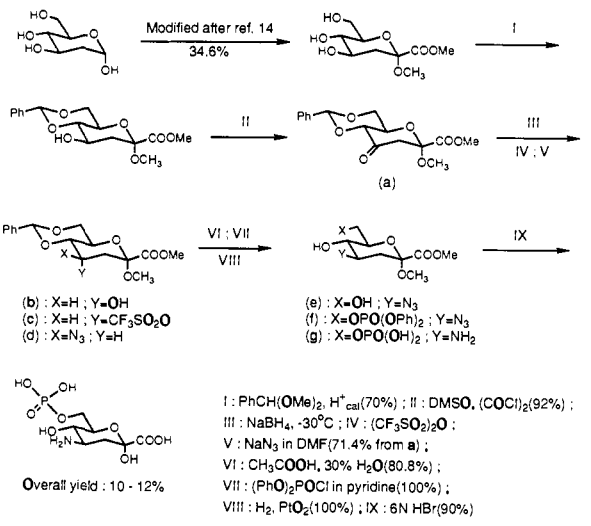
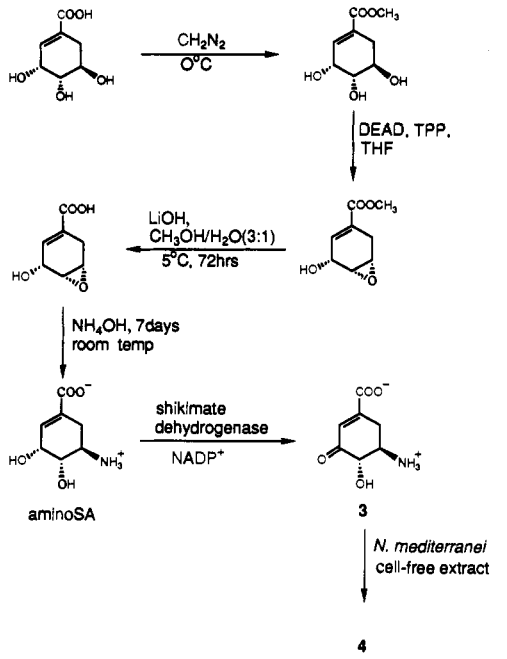
Scheme II



drolyzes glutamine, generating in the active site a molecule of ammonia. This forms a Schiff's base with erythrose 4-phosphate (E-4-P); condensation with phosphoenolpyruvate (PEP) then gives 3,4-dideoxy-4-amino-D-*arabino*-heptulosonic acid 7-phosphate (aminoDAHP, **2**).[13] Cyclization and dehydration, either by the normal shikimate pathway enzymes or by a separate set of enzymes, then produce 5-deoxy-5-amino-3-dehydroshikimic acid (aminoDHS, **3**), which aromatizes to **4**. We now present experimental evidence in support of this pathway.

2 was synthesized by the route shown in Scheme II. The synthesis largely follows that of Frost and Knowles[14] for DAHP, but with the introduction of nitrogen at C-4 by an S_N2 displacement of a triflate following inversion of configuration at that center. Samples of **2**[15] (0.3 mM) were incubated for 36 h at 28 °C with crude, undialyzed cell-free extracts (18–24 mg of protein/mL) of the **1** producer, *Nocardia mediterranei* S 699 (ATCC 21789), in 0.05 M potassium phosphate buffer, pH 7.4, in the presence of NAD^+ (0.3 mM), $CoCl_2$ (1.5 mM), and phenylmethanesulfonyl fluoride (PMSF) (1 mM). At the end of the incubation, 200 μg of [7-^{13}C]-**4** (90% ^{13}C) was added, and 1 h later the **4** was reisolated by EtOAc extraction, silylated, and analyzed by GC–MS for its ^{13}C enrichment. From this isotope dilution analysis it was determined that a typical incubation produced about 232 μg of **4** compared to 24 μg produced in a control containing all the ingredients except **2**. This corresponds to 45% conversion of the added **2** into **4**. No **4** was detectible in incubations carried out at 4 °C or ones from which NAD^+ and $CoCl_2$ or PMSF had been omitted. Incubations in which **2** had been replaced by DAHP (0.32 mM) with or without glutamine (0.68 mM) produced the same amount of **4** as the controls without **2**.[16] It follows from these data that **2** is an efficient precursor of **4** in *N. mediterranei* but that it is not formed from DAHP under the experimental conditions.

Scheme III



To test the proposed pathway further, we prepared **3** from shikimic acid as shown in Scheme III. 5-Deoxy-5-amino-shikimic acid (aminoSA) was synthesized[17] by regiospecific ring opening of a 4,5-epoxide[18] to give the product with the desired 5*R* configuration. AminoSA was a good substrate for shikimate dehydrogenase and could be oxidized preparatively with a cell-free extract[19] of *Escherichia coli* AB 2834/pIA321,[20] a genetically engineered overproducer of shikimate dehydrogenase, in the presence of $NADP^+$ and a $NADP^+$-regenerating system (oxidized glutathione, glutathione reductase). **3** was isolated as a white powder[21] by anion exchange chromatography (Bio-Rad AG 1X8, formate) followed by freeze-drying. In this form the compound was reasonably stable; surprisingly, it was not reduced by $NaBH_4$. Acid- or base-catalyzed decomposition produced exclusively protocatechuic acid, with no trace of **4** (<1%) detectible.

3 (0.58 mM) was incubated with a cell-free extract of *N. mediterranei* S 699 in 0.1 M Tris buffer, pH 7.5, at 28 °C.

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Subsequent isotope dilution analysis demonstrated 95% conversion of **3** into **4** within 10 h. Under similar conditions, aminoSA gave only 0.9% conversion into **4**.

The results reported here leave little doubt that AHBA, the precursor of mC_7N units, is produced by the proposed new variant of the shikimate pathway shown in Scheme I. Efforts are now underway to purify and characterize the enzymes catalyzing this new metabolic pathway and to establish their structural and evolutionary relationships to the normal shikimate pathway enzymes.

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Direct Evidence of Excited-State Intramolecular Proton Transfer in 2′-Hydroxychalcone and Photooxygenation Forming 3-Hydroxyflavone

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2′-Hydroxychalcones are widely distributed in plants and are precursors in vivo for different classes of flavonoids and isoflavonoids.[1,2] There have been several attempts to determine if 2′-hydroxychalcones are true intermediates in the biosynthesis of flavonoids.[3] Numerous reports have focused on studies of the photocyclization of 2′-hydroxychalcones, forming 4-flavanones in polar solvents.[4-6] However, to our knowledge, the relaxation dynamics of the excited 2′-hydroxychalcone have not been reported. Both NMR studies and semiempirical calculations indicate that the intramolecularly hydrogen-bonded species of 2′-hydroxychalcone (2HC, structure a in Figure 1) should dominate in the ground state. The 12.5 ppm downfield shift of the hydroxyl proton peak in benzene is indicative of the intramolecular hydrogen bond. The configuration of a is similar to *o*-hydroxybenzaldehyde types of excited-state intramolecular-proton-transfer (ESIPT) molecules.[7] Therefore, exploring the possibility of intramolecular proton transfer is intriguing and may provide valuable mechanistic information about the photochemistry of 2HC. In this communication we report the first observation of ESIPT for 2HC and its photooxygenation in nonpolar solvents, forming a well-known ESIPT molecule, 3-hydroxyflavone.

Figure 2 shows the absorption and photolysis-time-dependent emission of 2HC in aerated *n*-hexane at room temperature. For each measurement a fresh sample solution was prepared in the dark, and the emission was collected from the first six shots under the minimum excitation energy in order to avoid photodecomposition.[8] The maxima of the first and second absorption bands are ~354 and 314 nm with absorption extinction coefficients of $\sim 1.0 \times 10^4$ and 2.5×10^4 L^{-1} cm^{-1}, respectively (Figure 2A). When we utilized a red-sensitive diode array coupled with laser excitation (the third harmonic, 355 nm, of a Nd:YAG laser), a weak and broad emission with a maximum at ~635 nm was observed (Figure 2Ba). The fluorescence yield, Φ_f, is estimated to be $\sim 10^{-5}$ in *n*-hexane. Therefore, attempts to measure this emission using a commercially available fluorometer were unsuccessful. This emission is not quenched by oxygen, and its lifetime is beyond the limit of the resolution of the intensified detector (≪5 ns). Since the emission intensity is linearly proportional to the excitation energy and the prepared 2HC concentration, the possibility of the emission originating from a dimer or excimer of 2HC has also been excluded. The possibility that the observed fluorescence results from an impurity has been ruled out by employing several different methods of purification for 2HC. The intensity of the 635-nm emission is identical for all solutions prepared. In addition, although obtaining the excitation profile for this emission is not possible, no 635-nm emission was observed when the excitation wavelength was tuned from 500 to 590 nm, the likely absorption region of an impurity. We therefore conclude that the 635-nm emission originates from the $S'_1 \rightarrow S'_0$ (prime indicates the tautomer state) transition, where S'_1 is populated through $S_0 \rightarrow S_1$ absorption of conformer a followed by rapid ESIPT (Figure 1).

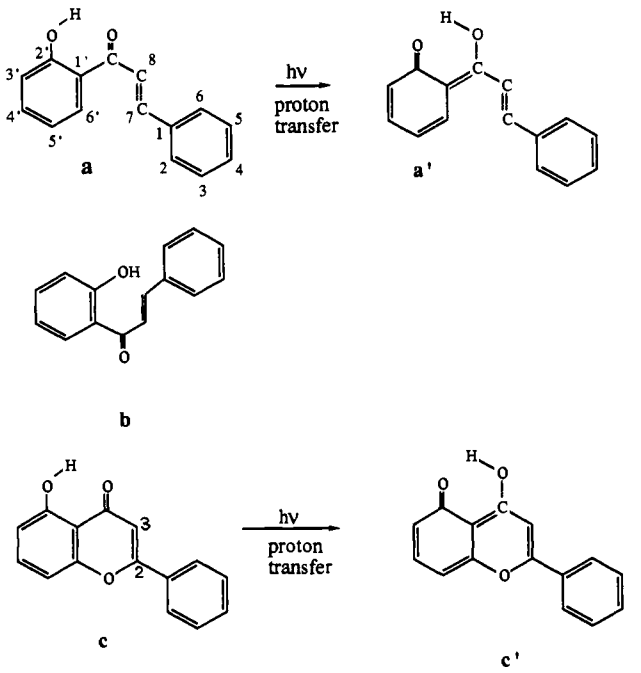

Figure 1. Structure of a, 2HC (normal, closed form); a′, 2HC (tautomer form); b, 2HC (normal, open form); c, 5-hydroxyflavone (normal form); and c′, 5-hydroxyflavone (tautomer form).

This observation is consistent with our earlier proposal that the tautomer emission wavelength can be qualitatively predicted using the simple Huckel approach by counting the number of nonaromatic conjugated double bonds of the tautomer.[9] For the case of 5-hydroxyflavone (Figure 1c) the tautomer species (c′) has five conjugated double bonds and exhibits an emission maximum at ~670 nm, which is ~3000 cm^{-1} lower in energy than that of 5-hydroxyflavanone, in which the C_2–C_3 double bond is hydrogenated. Since the 2HC tautomer (a′) has a similar structure and the same number of nonaromatic conjugated double bonds as 5-hydroxyflavone, the assignment of the 635-nm band to tautomer emission can be rationalized.

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