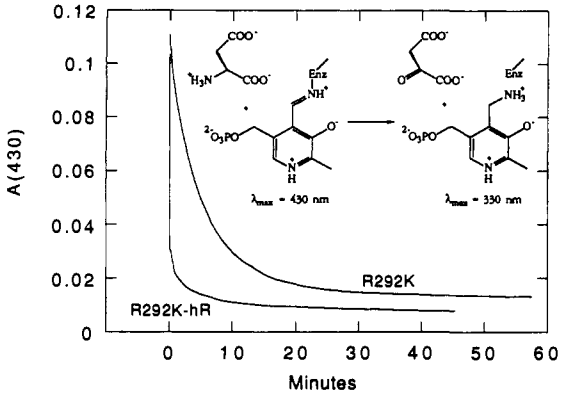

Figure 2. Single turnover reactions of R292K and *O*-methylisourea-treated R292K (R292K-hR) with aspartate. Enzyme activity was assayed in 100 mM MOPS/50 mM KCl at pH 7.0 using 1.0 mM aspartate and the coupling system reported in ref 9. The graph shows the decrease in absorbance at 430 nm due to the conversion of the pyridoxal phosphate cofactor to pyridoxamine phosphate, which absorbs at 330 nm.

At pH 7.5, the absorbance spectra of wild type AATase (WT) and the R292K mutant are identical. At this pH the PLP cofactor of AATase has absorbance peaks at 360 (unprotonated form) and 430 nm (protonated form). Since these peak positions are sensitive to local conformational changes,[9] it is likely that the conformation of the active site is not significantly perturbed by the mutation. Catalytic competence is greatly reduced, however, as seen by an increase in the K_m values for both substrates and a large decrease in k_{cat} (Table I).

MIU has been used previously to convert the lysine side chains of proteins to homoarginine.[10] Engler et al. have reported the conversion of an arginine in epidermal growth factor to homoarginine using the general procedure applied here.[3c] Nearly all lysines in an MIU-treated protein are modified, but guanidinated proteins are usually stable and possess nearly the same activity as unmodified enzymes. This is because lysine residues are typically found on the surface of proteins, and both amino and guanidino groups are positively charged at neutral pH. Modification of R292K with MIU should result in the guanidination of Lys 292 as well as the 17 surface lysines present per monomer. Lys 258, the catalytic base of AATase, should be protected because it is in a base-stable aldimine linkage with the PLP cofactor.

Optimal conditions for the reaction of both WT and R292K are reported in Figure 1. A significant amount of precipitate formed over the 4 day reaction time, but 63% of R292K and 41% of WT were recovered. Amino acid analysis of the R292K-hR sample showed that 78% of the lysine residues in R292K were modified. To assay the extent of modification specifically at Lys 292, single turnover experiments were carried out as shown in Figure 2. The rate constant for transamination of 1 mM aspartate by R292K is 0.0029 ± 0.0003 s^{-1}. Under identical conditions, 75% of the MIU-treated R292K reacts in the mixing time (k_{obsd} > 0.14 s^{-1}), while the remaining 25% turns over more slowly. This indicates that at least 75% of Lys 292 is converted to homoarginine under the conditions employed.

A comparison of the steady-state kinetic parameters for WT and modified WT shows a slight decrease in WT activity upon modification, due principally to a 3-fold decrease in the value of k_{cat} (Table I).[11] In contrast, R292K-hR shows a 100-fold increase in the value of k_{cat} over unmodified R292K, up to 60% that of the modified WT. The values for k_{cat}/K_m for R292K-hR are 100–300 times greater than those for R292K. R292K-hR, while less active than WT, is the most active position 292 AATase variant produced to date.

Acknowledgment. This work was supported by NIH Grant GM-35393, a gift from the Warner-Lambert Company, and an NIH postdoctoral fellowship awarded to P.W.W. Amino acid analyses were performed by the Protein Structure Laboratory at the University of California at Davis.

Registry No. AATase, 9000-97-9; Asp, 56-84-8; αKG, 328-50-7; Arg, 74-79-3; Lys, 56-87-1; homoarginine, 156-86-5.

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Total Synthesis and Absolute Stereochemical Assignment of Gilvocarcin M

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Gilvocarcin M (**1**) shows the key structural features of a growing class of aryl *C*-glycoside antibiotics[1] which share a common aromatic nucleus, 6*H*-benzo[*d*]naphtho[1,2-*b*]pyran-6-one, to which various rare sugars are connected through a C–C bond. These compounds are attractive as synthetic targets because of the challenge presented by their unusual *C*-glycoside structures linked to the highly functionalized skeleta and because some of the members show significant antitumor activity with exceptionally low toxicity.[2,3] The reported synthetic endeavors, however, have addressed only the aglycon portion (i.e., defucogilvocarcin),[4] and the synthesis of the full structure of the natural product with the sugar moiety remains a challenging problem.[5,6] A total synthesis is also desirable because, although the relative stereochemistry

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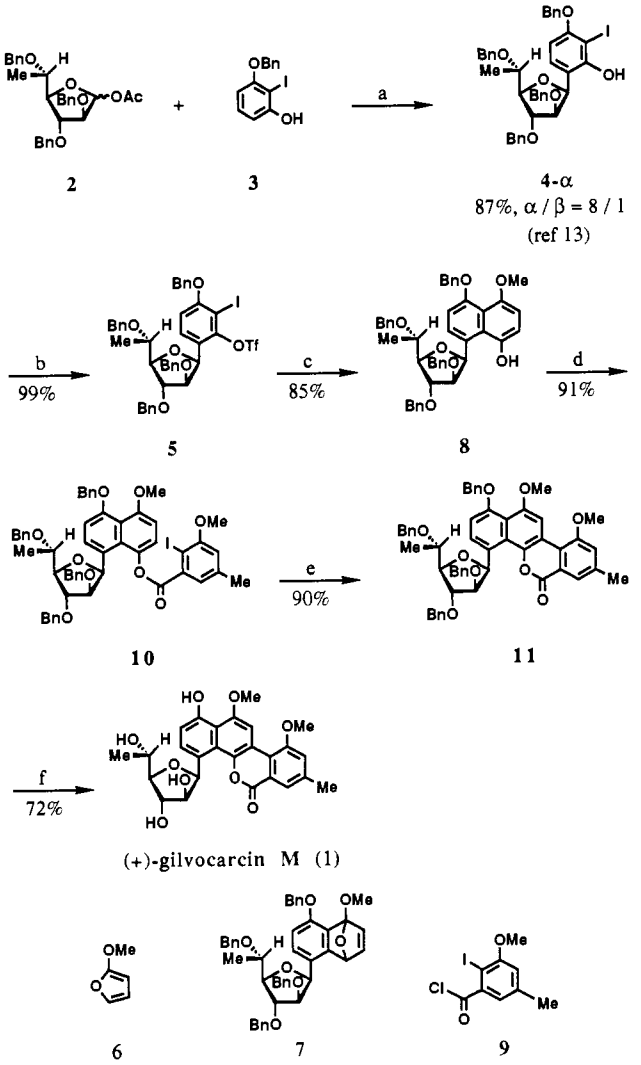
Scheme I[a]



[a] (a) Cp_2HfCl_2, $AgClO_4$/4-Å molecular sieves, CH_2Cl_2, −78 → −20 °C; (b) Tf_2O, $^iPr_2NEt/CH_2Cl_2$, −78 °C, 30 min; (c) **6**, *n*-BuLi/THF, −78 °C, 15 min; (d) **9**, $^iPr_2NEt$, cat. DMAP/THF, room temperature, 2 h; (e) $(Ph_3P)_2PdCl_2$, NaOAc/DMA, 125 °C, 6 h; (f) H_2, Raney Ni/EtOH, room temperature, 3 days.

of **1** has been established on the basis of X-ray crystallography, the absolute configuration remains to be defined.[2e,f] The L-fucose-based structure **1**, drawn in the original paper,[2f] has been accepted without proof.[7] In this paper we record the first total synthesis of (+)-gilvocarcin M (**1**) and thereby establish that the natural product is the enantiomer of that depicted in **1**. The synthetic route is exceedingly concise by virtue of two selective conversions (**2** + **3** → **4** and **5** + **6** → **8**) (Scheme I).

The acetate **2** was prepared from L-fucose,[8] and its conversion to an aryl *C*-glycoside was examined. This step is a major hurdle in the synthesis of **1**, since *C*-glycoside bond formation must be achieved in the 1,2-cis and 1,4-cis sense, which is sterically unfavorable. This contrasteric C–C-bond formation was cleanly effected by employing our glycosidation promoter, Cp_2HfCl_2–$AgClO_4$.[9] Thus, the glycosyl acetate **2**[10] (1 equiv) and the iodophenol **3**[11] (1.2 equiv) were treated with Cp_2HfCl_2–$AgClO_4$ (2 equiv each) in CH_2Cl_2 at −78 to −20 °C to obtain the *C*-glycoside **4** in 87% yield (α/β = 8/1).[12-14] Other promoters led to a decreased or reversed stereoselectivity ($SnCl_4$, α/β = 2.2/1; $BF_3{\cdot}OEt_2$, α/β = 1/1.8).

Another notable feature here is that aryl *C*-glycoside formation took place cleanly at the position ortho to the unprotected phenolic hydroxyl group.[9,14] This complete regiochemical control in the aromatic substitution was gratifying in view of the planned benzyne cycloaddition for constructing the naphthalene skeleton.[15] Accordingly, phenol **4**-α was converted to the corresponding triflate **5** quantitatively (Tf_2O, $^iPr_2NEt/CH_2Cl_2$, −78 °C), which was treated with *n*-BuLi (2 equiv) in THF at −78 °C in the presence of 2-methoxyfuran (**6**, 3 equiv). Sequential benzyne formation, [4 + 2] cycloaddition with the furan, and aromatization of the primary adduct **7** occurred smoothly to give the naphthol **8** (R_f 0.29 on silica gel TLC, hexane/Et_2O = 1/1) in 85% yield. A small amount of the regioisomeric adduct (9%; R_f 0.51) was also isolated. The observed regioselectivity can be attributed to the polar effect of the benzyloxy substituent, which controls the mode of cycloaddition.[16]

The final stage of the synthesis consisted of construction of the tetracyclic system, and this was achieved by the Pd-catalyzed internal cyclization approach developed by Martin et al.[4b] Acylation of **8** with the acid chloride **9**[17] gave the ester **10** (91% yield), which upon treatment with $(Ph_3P)_2PdCl_2$ (25 mol %) and NaOAc (3 equiv) in *N*,*N*-dimethylacetamide at 125 °C effected intramolecular biaryl coupling to produce the tetracycle **11** in 90% yield. Removal of the four benzyl groups by catalytic hydrogenolysis (H_2, Raney Ni/EtOH, 1 atm) gave (+)-gilvocarcin M (**1**) in 72% yield (mp 246–249 °C dec, lit.[2d] mp 245–248 °C dec). The synthetic material exhibited physical properties identical with those found for the natural product (TLC mobilities, 400-MHz ^1H NMR, 100-MHz ^{13}C NMR, IR, HRMS, UV). The sign of the optical rotation, however, was opposite to the one reported in the literature; $[\alpha]^{23}_D$ +209° (*c* 0.21, DMSO) [lit.[2d] $[\alpha]^{20}_D$ −209° (*c* 0.2, DMSO)]. *Thus, natural gilvocarcin M is the enantiomer of* ***1***.

The present synthesis is notable on three counts: (1) brevity (six steps), (2) high yield (38% overall from **2**), and (3) high regio- and stereoselectivity. Since the structure of **1** possesses all the essential elements of the gilvocarcin–ravidomycin class of antibiotics, the present synthetic approach will allow preparation of various congeners. The total synthesis of the natural enantiomer is in progress and will be reported shortly.

Acknowledgment. We are grateful to Dr. H. Saito, Kyowa Hakko Co., for a sample of **1**. Financial support from the Ministry of Education, Science and Culture of Japan [Grant-in-Aid for

(7) In ref 2f the authors stated in the text that D-fucose was assumed to be the sugar moiety, whereas the L-sugar was depicted. The latter enantiomer has been conventionally accepted. See also refs 2e and 2g.

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(12) All new compounds have been fully characterized by ^1H NMR, ^{13}C NMR, IR, and HRMS and/or combustion analyses.

(13) Note that the isomer depicted is the α-anomer since the L-series sugar is represented.

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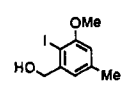

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Scientific Research on Priority Area (No. 03215229)] is gratefully acknowledged.

Supplementary Material Available: Analytical data including ^1H NMR, ^{13}C NMR, IR, $[\alpha]_D$, HRMS, and combustion data for the key compounds, **4**-α, **4**-β, **8**, and **1** (3 pages). Ordering information is given on any current masthead page.

Low-Temperature Growth of the Infinite Layer Phase of $SrCuO_2$ by Pulsed Laser Deposition

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The products obtained from conventional solid-state synthetic reactions are often limited to the high-temperature thermodynamic ones,[1,2] and thus there has been considerable interest in developing new low-temperature approaches that might provide access to novel phases and materials. For example, several groups have described the use of low-temperature reactive fluxes to prepare new metal–chalcogenide phases,[3,4] and other researchers have investigated the low-temperature synthesis of inorganic solids via the controlled decomposition of molecular precursors.[5] Herein, we report a strikingly different approach for low-temperature solid-state synthesis based on pulsed laser deposition (PLD), and we use this method to prepare the tetragonal infinite layer phase of $SrCuO_2$. The tetragonal phase of $SrCuO_2$ is an important target since it represents the parent structure of the high-temperature copper oxide superconductors; however, it is inaccessible by conventional high-temperature synthetic routes.

PLD is a well-established technique for the preparation of thin film materials.[6-9] It involves ablation of a target material with a high-energy pulsed laser, and deposition of the evaporated target material onto a substrate to yield a thin film product. There are several features of the ablation and deposition processes that make PLD ideally suited for the synthesis of new materials, including the following: (1) material is congruently evaporated from the target during rapid laser heating; (2) growth of a crystalline product can be carried out at temperatures significantly lower than those of conventional solid-state reactions; (3) the substrate can be chosen to enforce the growth of a specific structural phase; and (4) sequential multitarget evaporation can be used to prepare complex layered structures with atomic-level control.[6-8] An indication of the potential of PLD for materials synthesis has been the growth of high-quality, epitaxial films of copper oxide superconductors, because these materials are compositionally complex and melt incongruently.[7,8] There are few studies, however, that have utilized the unique characteristics of PLD for new materials synthesis.[9]

Our PLD experiments were carried out in a stainless steel vacuum chamber that is evacuated with a turbomolecular pump (Figure 1). The $SrCuO_2$ target material was prepared by conventional high-temperature methods from a 1:1 mixture of $SrCO_3$ and CuO.[10] This preparative route yields an orthorhombic phase

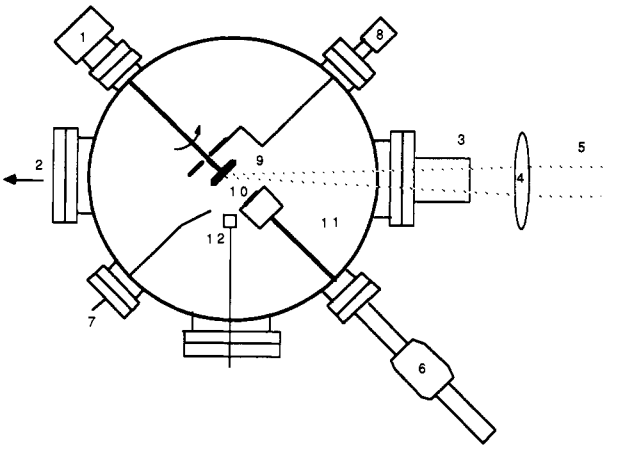

Figure 1. Schematic view of the PLD chamber. Major components: (1) target rotation feedthrough, (2) turbomolecular pump, (3) quartz window, (4) focus lens, (5) excimer laser, (6) substrate manipulator, (7) gas inlet, (8) substrate shutter, (9) target, (10) substrate, (11) substrate heater, (12) quartz crystal thickness monitor.

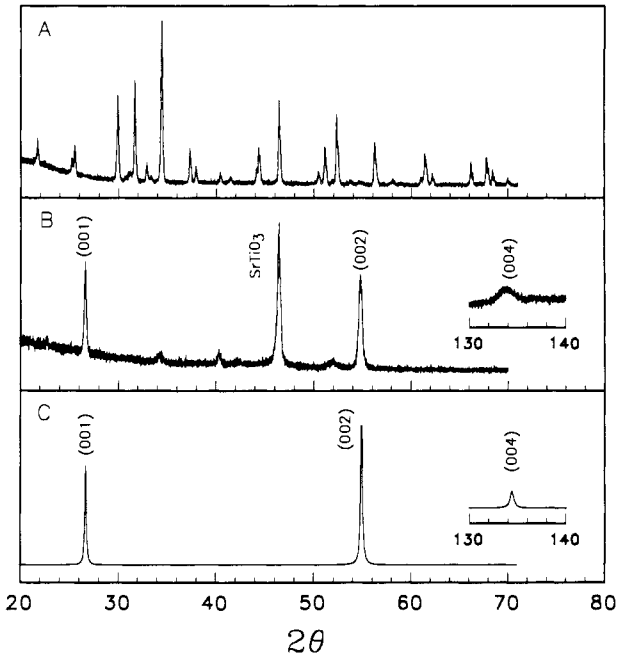

Figure 2. Experimental X-ray diffraction patterns for (A) the orthorhombic $SrCuO_2$ target and (B) a 5000-Å $SrCuO_2$ film prepared by PLD. A simulated diffraction pattern for *c*-axis oriented tetragonal $SrCuO_2$ is shown in part C.

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(10) Stoichiometric amounts of CuO (99.999%, Johnson Matthey) and $SrCO_3$ (99.999%, Johnson Matthey) were ground, heated at 800 °C for 12 h, reground, and heated at 900 °C for 24 h. Finally, the resulting polycrystalline powder was pressed into a pellet and sintered at 950 °C.

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