1.1 mmol), and the solution stirred for 5 min. The resulting solution was transferred by cannula with rinsing (toluene, 2 mL) into a carefully evacuated and argon filled stainless-steel autoclave. The autoclave was heated to 90 °C and subsequently pressurized with H_2/CO (1/1, 20 bar). After stirring the mixture for 48 h at this temperature the autoclave was cooled rapidly to 20 °C and depressurized. The reaction solution was filtered through a small pad of silica with *tert*-butyl methyl ether (50 mL). After evaporation of the solvent the crude product was analyzed by NMR spectroscopy to determine the diastereomer ratio (96:4). Subsequent column chromatography on silica (solvent: petrolether/*tert*-butyl methyl ether, 9/1) provided the unsaturated ester (±)-**3** (391 mg, 0.75 mmol) as a highly viscous oil.

[6] All compounds were characterized by ^{1}H, ^{13}C, ^{31}P NMR spectroscopies and elemental analysis. Selected physical data for (±)-**3**: ^{1}H NMR (300.133 MHz, $CDCl_3$): $\delta = 0.66 - 0.75$ (m, 9H, $3CH_3$), 1.14 (t, $J = 7.1$ Hz, 3H, CH_3CH_2), 1.61 (s, 3H, CH_3), 1.73 – 2.01 (m, 4H), 4.03 (q, $J = 7.1$ Hz, 2H, CH_2), 4.72 (dd, $J = 7.6$ Hz, 3.7 Hz, 1H), 6.58 (m_c, 1H, CH-olefin), 6.79 (m_c, 1H, ArH), 7.11 – 7.16 (m, 11H, ArH), 7.23 (m_c, 1H, ArH), 7.99 (m_c, 1H, ArH); ^{13}C NMR (75.469 MHz, $CDCl_3$): $\delta = 12.21$, 13.34, 13.91, 17.93, 18.84, 29.38, 32.71, 34.28, 59.96, 81.87, 127.76, 128.01 (d, $J_{C,P} = 7.1$ Hz, 2C), 128.08 (d, $J_{C,P} = 7.2$ Hz, 2C), 128.25 (2C), 128.52, 130.10, 130.81 (2C), 133.53 (d, $J_{C,P} = 20.8$ Hz, 2C), 133.66 (d, $J_{C,P} = 21.0$ Hz, 2C), 133.93, 137.78 (d, $J_{C,P} = 12.5$ Hz), 137.81 (d, $J_{C,P} = 11.9$ Hz), 139.75 , 140.88 (d, $J_{C,P} = 28.2$ Hz), 165.92, 167.60; ^{31}P NMR (81.015 MHz, $CDCl_3$): $\delta = -2.9$; elemental analysis calcd for $C_{32}H_{37}O_4P$ (516.62): C 74.49, H 7.22; found: C 74.61, H 7.34. Selected physical data for (±)-**9**: ^{1}H NMR (300.133 MHz, $CDCl_3$): $\delta = 0.67 - 0.78$ (m, 9H, $3CH_3$), 0.97 (m, 1H, CH), 1.08 (m, 1H, CH), 1.43 (m, 2H, CH_2), 1.65 (m, 1H, CH), 1.80 (m, 1H, CH), 1.97 (s, 3H, CH_3), 2.10 – 2.20 (m, 2H, CH_2), 4.72 (dd, $J = 7.8$ Hz, 4.1 Hz, 1H), 6.82 (m_c, 1H, ArH), 7.10 – 7.20 (m, 11H, ArH), 7.26 (m_c, 1H, ArH), 8.01 (m_c, 1H, ArH); ^{13}C NMR (75.469 MHz, $CDCl_3$): $\delta = 13.40$, 18.08, 18.88, 21.04, 26.61, 29.20, 32.84, 34.01, 43.42, 81.55, 124.91, 127.76, 128.04 (d, $J_{C,P} = 6.8$ Hz, 2C), 128.12 (d, $J_{C,P} = 5.7$ Hz, 2C), 128.62, 130.08, 131.47 (2C), 133.52 (d, $J_{C,P} = 20.8$ Hz, 2C), 133.68 (d, $J_{C,P} = 21.0$ Hz, 2C), 133.88, 137.63 (d, $J_{C,P} = 10.3$ Hz), 137.96 (d, $J_{C,P} = 11.8$ Hz), 140.77 (d, $J_{C,P} = 27.9$ Hz), 165.97, 208.44; ^{31}P NMR (81.015 MHz, $CDCl_3$): $\delta = -2.9$; elemental analysis calcd for $C_{30}H_{35}O_3P$ (474.58): C 75.93, H 7.43; found: C 75.77, H 7.57.

[7] The relative configuration of the domino reaction products was determined at the aldeyde stage, since the stereochemistry-determining step is the hydroformylation.[8, 9, 11] This was confirmed by stepwise transformation of the known aldehyde (±)-**19** derived from olefin (±)-**1**[8] into both the domino hydroformylation-Wittig product (±)-**20** ($Ph_3P{=}CHCOMe$, CH_2Cl_2, RT, 2 d, 80%) and the domino hydroformylation-wittig-hydrogenation product (±)-**9** (0.7 mol% [Rh(H)(CO)(PPh_3)$_3$], H_2 (20 bar), toluene, 60 °C, 24 h, 62%).

[8] a) B. Breit, *Angew. Chem.* **1996**, *108*, 3021 – 3023; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2835 – 2837; b) B. Breit, *Liebigs Ann.* **1997**, 1841 – 1851.

[9] a) B. Breit, *Chem. Commun.* **1997**, 591 – 592; b) B. Breit, *Eur. J. Org. Chem.* **1998**, 1123 – 1134.

[10] R. W. Hoffmann, *Angew. Chem.* **1987**, *99*, 503 – 517; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 489 – 503.

[11] B. Breit, S. K. Zahn, *Tetrahedron Lett.* **1998**, *39*, 1901 – 1904.

A Concise Synthesis of Fumagillol**

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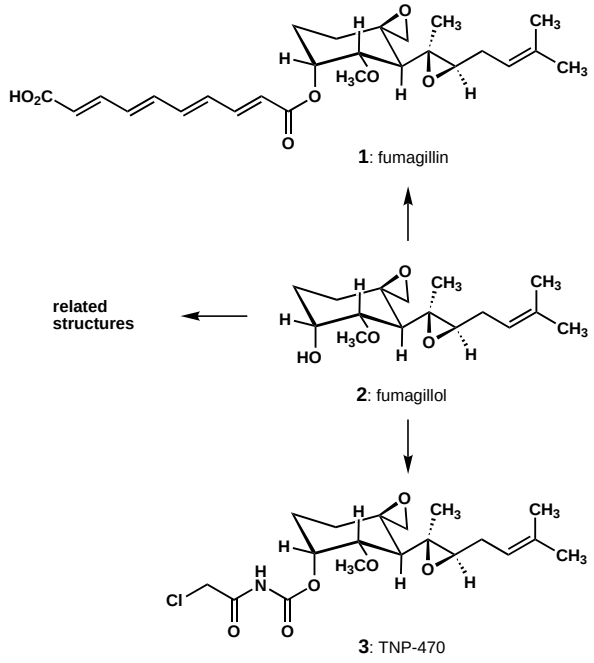
Fifty years ago Hanson and Eble reported that cultures of the fungus *Aspergillus fumigatus* inhibited *Staphylococcus aureus* 209 bacteriophage.[1] The active constituent was named fumagillin, and was soon renowned for its potent antiparasitic properties.[2] The meritorious studies of Tarbell and co-workers yielded insight into the chemical behavior of fumagillin and a hypothesis about its constitution.[3] An X-ray crystallographic analysis confirmed the outcome of the chemical degradation campaign and thus established the structures of fumagillin (**1**) and its saponification product fumagillol (**2**).[4] Each substance is distinguished by two



epoxide functions, one of which is highly reactive, and six contiguous stereocenters. Fumagillin presented itself as an attractive objective for research in organic synthesis because of its novel structure and utility as an amebicide.[5] In a landmark paper in 1972 Corey and Snider described their elegant studies that culminated in the first chemical synthesis of this natural product.[6, 7]

Interest in the biological properties of fumagillin experienced a renaissance when Judah Folkman and his group discovered that **1** and its semi-synthetic derivative TNP-470

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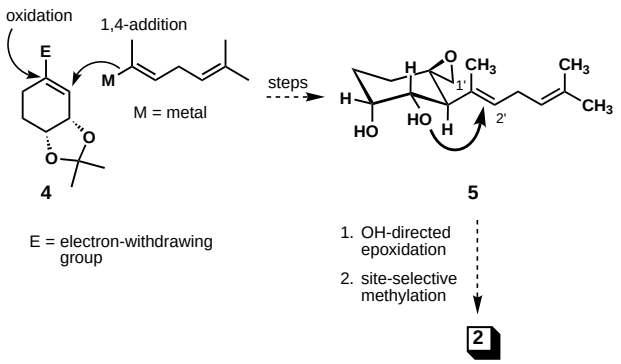
(**3**) have the remarkable capacity to inhibit the proliferation of endothelial cells in vitro and tumor-induced angiogenesis in vivo.[8] Since unregulated angiogenesis can cause the growth and metastasis of malignant tumors and inflammatory diseases, inhibitors of this process are of considerable interest as potential chemotherapeutic agents.[9] TNP-470 (**3**) is currently one of the most promising small molecule inhibitors of angiogenesis and is being evaluated in phase III clinical trials as a potential cancer drug. In recent work, fumagillin-derived affinity reagents allowed the identification of methionine aminopeptidase-2 (MetAP-2) as the molecular target of fumagillin's action.[10–12] Fumagillin may prevent the myristoylation of a key endothelial cell regulatory protein[9e, 13] by irreversibly inhibiting MetAP-2, a cobalt-containing metalloprotease that removes methionine residues from the N termini of proteins in a critical co-translational processing event.[14] Research in our laboratory sought a concise solution to the chemical problem posed by these structures. Herein we describe a 13-step synthesis of (±)-fumagillol (**2**), the immediate precursor of fumagillin (**1**) and TNP-470 (**3**), from abundant and inexpensive starting materials.

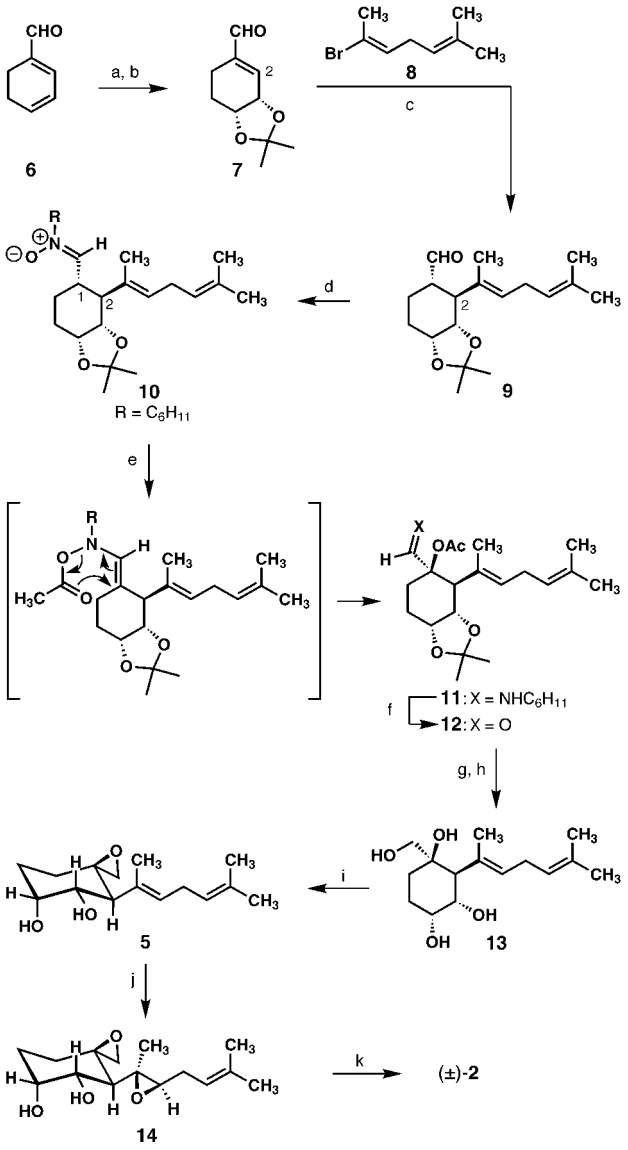
The essence of our strategy is a vicinal difunctionalization of the electron-deficient alkene of a compound of type **4** (E = electron-withdrawing group), after which a short reaction sequence could give rise to diol **5** (Scheme 1). Although **5**



Scheme 1. An approach to the synthesis of **2**, which features a vicinal difunctionalization of **4**.

bears two trisubstituted alkenes, the C1′–C2′ double bond is distinguished by its proximity to a free hydroxyl group. Our intention was to explore the feasibility of effecting a site- and diastereoselective hydroxyl-directed epoxidation of the C1′–C2′ olefin.[15] A selective methylation of the equatorial C-3 hydroxyl group, a reaction shown to be feasible by Corey and Snider,[6] would then complete a synthesis of **2**.

Our synthesis of (±)-**2** was guided by this general analysis and achieved by the pathway presented in Scheme 2. Upjohn dihydroxylation[16] of the more electron-rich γ,δ-double bond of 1,3-cyclohexadiene-1-carbaldehyde (**6**), a known substance[17] that can be prepared in bulk from acrolein and 1-diethylamino-1,3-butadiene,[18] afforded a diol (73% yield) that could be efficiently protected as isopropylidene ketal **7** (87% yield). By this sequence we could procure multi-gram quantities of enal **7**.



Scheme 2. Synthesis of (±)-**2**: a) OsO_4 (0.5 mol%), NMO · H_2O (2.5 equiv), 2-propanol, RT, 1 h, 73%; b) $(CH_3)_2CO$, *p*-TsOH (0.05 equiv), RT, 1 h, 87%; c) **8**, *t*BuLi (2.1 equiv), Et_2O, −78 °C, 1 h; then Li(2-thienyl)CuCN (1.2 equiv), 1 h; then **7**, $BF_3 \cdot OEt_2$ (1.0 equiv), Et_2O, 5 min, 46%; d) $C_6H_{11}NHOH$ (2.0 equiv), EtOH, $NaHCO_3$ (5.0 equiv), RT, 1 h; e) AcCl (1.0 equiv), Et_3N (1.0 equiv), Et_2O, 0 °C → RT, 0.5 h; f) AcOH, NaOAc, RT, 1 h, 51% from **9**; g) $LiAlH_4$ (6.0 equiv), Et_2O, 0 °C → RT, 1 h, 71%; h) 2.0 N HCl/THF (4/1), RT, 1 h, 81%; i) MsCl (2.0 equiv), 4-DMAP (0.2 equiv), Et_3N (5.0 equiv), CH_2Cl_2, −30 °C, 0.2 h; then 6.0 N NaOH (10.0 equiv), MeOH, RT, 0.5 h, 97%; j) [$VO(acac)_2$] (0.2 equiv), *t*BuOOH (3.0 equiv), benzene, RT, 5 min, 75% (4.3/1 ratio of epoxide diastereomers in favor of **14**); k) *t*BuONa (1.4 equiv), MeI (3.0 equiv), THF, [15]crown-5, RT, 2 h, 40% of (±)-**2** plus 40% recovered **14**. NMO = 4-methylmorpholine *N*-oxide, *p*-TsOH = *p*-toluenesulfonic acid, MsCl = methanesulfonyl chloride, DMAP = 4-dimethylaminopyridine, [$VO(acac)_2$] = vanadyl acetylacetonate.

We hoped that the *cis*-ring fusion of bicycle **7** would permit a diastereocontrolled bond formation at C-2.[19] Despite the presence of two electrophilic sites in enal **7**, the organocuprate reagent derived from vinylbromide **8**[20–22] reacted with **7** in a highly regio- and diastereoselective fashion in the presence of $BF_3 \cdot OEt_2$ and gave a 3/1 mixture of C-1 epimers in favor of aldehyde **9** (Table 1).[23] While the minor epimer could be

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Table 1. Selected physical properties of compounds **9**, **12**, and **2**.

9: TLC: $R_f = 0.50$ (silica gel, hexanes/EtOAc (5/1)); IR (film): $\tilde{\nu} = 1726\ cm^{-1}$ (C=O); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.43$ (d, $J = 3.5$ Hz, 1H; CHO), 5.30–5.26 (m, 1H; H-2′), 5.07–5.03 (m, 1H; H-4′), 4.28–4.26 (m, 1H; H-4), 3.98 (dd, $J = 4.8$, 9.2 Hz, 1H; H-3), 2.73–2.70 (m, 2H; H-3′), 2.41 (dd, $J = 9.3$, 11.9 Hz, 1H; H-2), 2.27–2.19 (m, 2H), 1.77–1.71 (m, 3H), 1.68 (s, 3H), 1.66 (s, 3H), 1.60 (s, 3H), 1.55 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 203.2$, 132.0, 128.8, 122.5, 108.4, 76.4, 72.8, 50.1, 49.8, 28.6, 28.5, 26.9, 26.2, 25.6, 25.5, 20.5, 17.7, 13.6; HR-MS (FAB): m/z calcd for $[M^+ + Na]$ 315.1936, found 315.1927.

12: TLC: $R_f = 0.26$ (silica gel, hexanes/EtOAc (5/1)); IR (film): $\tilde{\nu} = 1735\ cm^{-1}$ (C=O); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 9.33$ (s, 1H; CHO), 5.31–5.28 (m, 1H; H-2′), 5.06–5.02 (m, 1H; H-4′), 4.39 (dd, $J = 4.8$, 9.9 Hz, 1H; H-3), 4.33–4.31 (m, 1H; H-4), 2.73–2.70 (m, 2H; H-3′), 2.29 (d, $J = 9.9$ Hz, 1H; H-2), 2.27–1.25 (m, 4H), 2.16 (s, 3H; $C(O)CH_3$), 1.77 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H), 1.48 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 199.0$, 170.0, 132.3, 128.7, 122.1, 108.5, 87.3, 74.8, 72.4, 53.4, 50.2, 28.5, 27.1, 26.3, 25.6, 23.2, 21.8, 20.9, 17.8, 15.4; HR-MS (FAB): m/z calcd for $[M^+H]$ 351.2171, found 351.2181.

(±)-fumagillol (**2**): TLC: $R_f = 0.41$ (silica gel, CH_2Cl_2/acetone (3/1)); IR (film): $\tilde{\nu} = 3430\ cm^{-1}$ (OH); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 5.21–5.17$ (m, 1H; H-4′), 4.37–4.35 (m, 1H; H-4), 3.61 (dd, $J = 3.0$, 11.4 Hz, 1H; H-3), 3.48 (s, 3H; OCH_3), 2.92 (d, $J = 4.0$ Hz, 1H; exomethylene oxide H), 2.57 (t, $J = 6.5$ Hz, 1H; H-2′), 2.52 (d, $J = 4.0$ Hz, 1H; exomethylene oxide H), 2.39–2.33 (m, 2H; H-3′), 2.3–1.3 (m, 5H), 1.91 (d, $J = 11.4$ Hz, 1H; H-2), 1.73 (s, 3H), 1.64 (s, 3H), 1.20 (s, 3H; CH_3-1′); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 134.9$, 118.5, 80.9, 64.0, 61.2, 59.8, 58.5, 56.5, 50.7, 47.0, 28.5, 27.4, 26.5, 25.7, 18.0, 13.9; HR-MS (FAB): m/z calcd for $[M^+ + Na]$ 305.1729, found 305.1734.

converted into **9** on treatment with *t*BuOK in *t*BuOH, both stereoisomers could be converted into α-acetoxyaldehyde **12** (see below). Gratifyingly, the C-2 epimer of **9** was not observed. The cyclic protecting group of enal **7** thus dictated the stereochemical course of the crucial carbon–carbon bond formation.

Our efforts to introduce the needed C-1 oxygen atom via the enolate of aldehyde **9** encountered significant resistance. However, treatment of *N*-cyclohexylnitrone **10**, derived in one step from aldehyde **9**, with acetyl chloride and triethylamine yielded α-acetoxy-*N*-cyclohexylimine **11**, presumably via a [3,3] sigmatropic isomerization of a transitory *N*-vinyl-*O*-acetylhydroxylamine[24] as shown in Scheme 2. While imine **11** could be isolated, it was convenient to convert crude **11** into aldehyde **12** by hydrolysis with mild aqueous acid. This under-utilized method, developed by Cummins and Coates[24] for creating α-acetoxycarbonyl compounds, provided a simple solution to the nontrivial problem of introducing the requisite oxygen atom at C-1 in a diastereocontrolled fashion. We could create compound **12** in good yield and diastereoselectivity by this procedure (51% over three steps; less than 5% of the C-1 epimer was isolated). It is difficult to advance a compelling argument to explain the observed diastereoselectivity, but we note that the acetoxy group in **12** is situated on the convex face of the bicyclic framework.

The path to **2** from compound **12** was a straightforward one. After reduction of **12** with lithium aluminum hydride, acid-catalyzed hydrolysis of the isopropylidene ketal provided tetraol **13**. Although the primary alcohol moiety of **13** is flanked by a fully substituted carbon atom, it could be converted selectively into the corresponding methanesulfonate ester. Direct treatment of the reaction mixture with methanolic sodium hydroxide solution then afforded spiroepoxide **5**. We hoped to exploit a documented side-chain conformational preference[6] and the close spatial relationship between the free hydroxyl at C-3 and the interior side-chain double bond to achieve the introduction of the remaining epoxide function of fumagillol. When **5** was subjected to Sharpless's vanadium-based epoxidation protocol,[25] **14** was isolated in 61% yield (75% yield of a 4.3/1 mixture of diastereomers in favor of **14**).[26] Finally, exposure of **14** to *t*BuONa and iodomethane[27] resulted in the formation of (±)-**2**, identical in all respects, except optical rotation, to a sample derived from natural fumagillin (**1**).

In summary, we have described a chemical synthesis of **2** by a pathway that is distinguished by its brevity and reliance on relatively simple starting materials and reagents. We are currently adapting our strategy to the production of fumagillol in enantiomerically pure form. In passing, we note that the hypothetical construct **4** wherein $E = CO_2Me$ (Scheme 1) is available in optically active form[28] and can be converted into enal **7** (Scheme 2) through a two-stage reduction/oxidation sequence. The convergent nature of the strategy described herein may facilitate the synthesis of manifold fumagillin-like small molecules that may show utility as inhibitors of angiogenesis.

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[1] F. R. Hanson, E. Eble, *J. Bact.* **1949**, *58*, 527–529.
[2] a) M. C. McCowen, M. E. Callender, J. F. Lawlis, Jr., *Science* **1951**, *112*, 202–203; b) H. Katznelson, C. A. Jamieson, *Science* **1952**, *115*, 70–71; c) J. H. Killough, G. B. Magill, R. C. Smith, *Science* **1952**, *115*, 71–72.
[3] a) D. S. Tarbell, R. M. Carman, D. D. Chapman, S. E. Cremer, A. D. Cross, K. R. Huffman, M. Kunstmann, N. J. McCorkindale, J. G. McNally, Jr., A. Rosowsky, F. H. L. Varino, R. L. West, *J. Am. Chem. Soc.* **1961**, *83*, 3096–3113, and references therein; b) J. R. Turner, D. S. Tarbell, *Proc. Natl. Acad. Sci. USA* **1962**, *48*, 733–735.
[4] N. J. McCorkindale, J. G. Sime, *Proc. Chem. Soc.* **1961**, 331.
[5] For early attempts to achieve a synthesis of fumagillin, see a) S. T. Young, J. R. Turner, D. S. Tarbell, *J. Org. Chem.* **1963**, *28*, 928–932; b) G. Büchi, J. E. Powell, Jr., *J. Am. Chem. Soc.* **1970**, *92*, 3126–3133.
[6] a) E. J. Corey, B. B. Snider, *J. Am. Chem. Soc.* **1972**, *94*, 2549–2550; b) B. B. Snider, PhD Thesis, Harvard University, **1973**.
[7] For a recent 26-step synthesis of (−)-fumagillol, see D. Kim, S. K. Ahn, H. Bae, W. J. Choi, H. S. Kim, *Tetrahedron Lett.* **1997**, *38*, 4437–4440.
[8] D. Ingber, T. Fujita, S. Kishimoto, K. Sudo, T. Kanamaru, H. Brem, J. Folkman, *Nature* **1990**, *348*, 555–557.
[9] a) J. Folkman, *New Engl. J. Med.* **1971**, *285*, 1182–1186; b) J. Folkman, *New Engl. J. Med.* **1995**, *333*, 1757–1763; c) J. Folkman, *Nat. Med.* **1995**, *1*, 27–31; d) A. Giannis, F. Rübsam, *Angew. Chem.* **1997**, *109*, 606–609; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 588–590; e) J. Taunton, *Chem. Biol.* **1997**, *4*, 493–496.
[10] N. Sin, L. Meng, M. Q. W. Wang, J. J. Wen, W. G. Bornmann, C. M. Crews, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 6099–6103.
[11] E. C. Griffith, Z. Su, B. E. Turk, S. Chen, Y.-H. Chang, Z. Wu, K. Biemann, J. O. Liu, *Chem. Biol.* **1997**, *4*, 461–471.
[12] Very recently, Clardy and co-workers reported an X-ray crystal structure of human MetAP-2 covalently bound by fumagillin, see S. Liu, J. Widom, C. W. Kemp, C. M. Crews, J. Clardy, *Science* **1998**, *282*,

1324 – 1327. See also W. T. Lowther, D. A. McMillen, A. M. Orville, B. W. Matthews, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12153 – 12157.
[13] For a recent review of protein myristoylation, see J. A. Boutin, *Cell. Signalling* **1997**, *9*, 15 – 35.
[14] T. Yoshida, Y. Kaneko, A. Tsukamoto, K. Han, M. Ichinose, S. Kimura, *Cancer Res.* **1998**, *58*, 3751 – 3756.
[15] For a scholarly review of substrate-directable chemical reactions, see A. H. Hoveyda, D. A. Evans, G. C. Fu, *Chem. Rev.* **1993**, *93*, 1307 – 1370.
[16] V. Van Rheenen, R. C. Kelly, D. Y. Cha, *Tetrahedron Lett.* **1976**, 1973 – 1976.
[17] W. Langenbeck, O. Godde, L. Weschky, R. Schaller, *Ber. Dtsch. Chem. Ges.* **1942**, *75*, 232 – 236.
[18] 1-Diethylamino-1,3-butadiene can be prepared on a large scale in one step from diethylamine and crotonaldehyde, see S. Hünig, H. Kahanek, *Chem. Ber.* **1957**, *90*, 238 – 245.
[19] Woodward's landmark synthesis of reserpine provides instructive, early instances of this tactic, see R. B. Woodward, F. E. Bader, A. J. Frey, R. W. Kierstead, *Tetrahedron* **1958**, *2*, 1 – 57.
[20] Vinyl bromide **8** was prepared by a slight modification of the excellent one-flask procedure of Corey et al., see E. J. Corey, J. Lee, B. E. Roberts, *Tetrahedron Lett.* **1997**, *38*, 8915 – 8918. See also E. J. Corey, J. P. Dittami, *J. Am. Chem. Soc.* **1985**, *107*, 256 – 257.
[21] For the use of Li(2-thienyl)CuCN, see B. H. Lipshutz, M. Koerner, D. A. Parker, *Tetrahedron Lett.* **1987**, *28*, 945 – 948.
[22] For reviews of the conjugate addition chemistry of organocuprates, see a) B. H. Lipshutz, S. Sengupta, *Org. React.* **1992**, *41*, 135 – 631; b) J. A. Kozlowski in *Comprehensive Organic Synthesis*, *Vol. 1* (Eds.: B. M. Trost, I. Fleming, S. L. Schreiber), Pergamon, New York, **1991**, pp. 169 – 198.
[23] Similar results were obtained when chlorotrimethylsilane was used in place of $BF_3 \cdot OEt_2$, followed by fluoride-induced cleavage of the crude enol silyl ethers.
[24] a) C. H. Cummins, R. M. Coates, *J. Org. Chem.* **1983**, *48*, 2070 – 2076; b) R. M. Coates, C. H. Cummins, *J. Org. Chem.* **1986**, *51*, 1383 – 1389.
[25] K. B. Sharpless, R. C. Michaelson, *J. Am. Chem. Soc.* **1973**, *95*, 6136 – 6137.
[26] While our studies were underway, a closely related transformation was described, see S. Amano, N. Ogawa, M. Ohtsuka, S. Ogawa, N. Chida, *Chem. Commun.* **1998**, 1263 – 1264.
[27] This is a slight modification of a procedure employed in Corey and Snider's pioneering synthesis of fumagillin (reference [6]).
[28] Structure **4** ($E = CO_2Me$) in optically active form is available from (−)-quinic acid (D. F. McComsey, B. E. Maryanoff, *J. Org. Chem.* **1994**, *59*, 2652 – 2654) or by Sharpless asymmetric dihydroxylation followed by isopropylidene ketal formation (Z.-M. Wang, K. Kakiuchi, K. B. Sharpless, *J. Org. Chem.* **1994**, *59*, 6895 – 6897).

Dynamic Light Scattering Evidence for a Ligand-Induced Motion between the Two Domains of Glucoamylase G1 of *Aspergillus niger* with Heterobivalent Substrate Analogues**

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Glucoamylases (GAs) catalyze the hydrolytic release of β-D-glucose from the nonreducing ends of starch and related oligo- and polysaccharides. Most GAs possess a starch-binding domain (SBD) separated from the catalytic domain (CD) by a glycosylated peptide linker of variable length.[1] Removal of the SBD reduces the activity of GA from *Aspergillus niger* on insoluble starch but not on soluble substrates.[2] We have previously shown that 6^{II}-thiopanose and its higher oligomers bind essentially to the SBD and modulate GA activity on starch.[3] This raised the possibility of an interaction between the CD and the SBD of GA, and these observations have suggested that a cooperativity of the two domains could be critical for optimal activity.[4] The only low resolution structural information available so far on the entire GA was obtained by scanning tunneling microscopy,[5] but the possible mobility of the two domains induced by substrate binding cannot be described by this technique. The three-dimensional structure of the CD of the GA from *Aspergillus awamori* X100 has been solved by X-ray crystallography,[6] while that of the SBD of GA isolated from *Aspergillus niger* has been recently determined by NMR spectroscopy.[7] Failure to crystallize the entire GA has repeatedly been observed and this is attributed to the inherent flexibility of the linker peptide that connects the two constitutive domains. Co-crystallization in the presence of a ligand targeted to both the CD and SBD may stabilize one conformer. Recently, closure of a flexible loop onto a substrate analogue has allowed the crystallization of a cellulase.[8]

Herein we describe the design and synthesis of high affinity probes that bind the CD and SBD of GA at the same time, and hence get new insights into the structure/activity relationships of GA. The structural variations of GA between free and bound states were monitored by quasi-elastic light

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