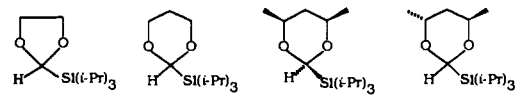
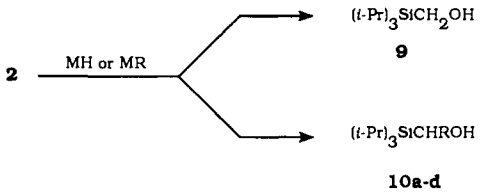
Also, GC analysis revealed that **2** was efficiently converted back to its precursors, **1** (81%, $CH_2(CH_2SH)_2$, BF_3·EE (0.16 equiv), $CHCl_3$ or 77%, $(CH_2)_3S_2SiMe_2$, BF_3·EE (0.4 equiv), CH_2Cl_2)[6a,13] and **3** (93%, $CH(OMe)_3$, TsOH or 100%, $CH(OMe)_3$, MeOH, Clay K 10).[14] Standard methodology (diol, TsOH, C_6H_6, reflux) produced the cyclic acetals (**5** (76%), **6** (72%)). For **6**, MMX calculations predict a strong preference for the $TIPS_{eq}$ chair conformation (>6 kcal/mol) which is revealed in its ^1H NMR through distinctly separated signals for each of the ring hydrogens and by vicinal coupling constants which are matched (±0.2 Hz) by calculation for this conformation. Thus, the reaction of **2** with a 60:40 meso/dl mixture of 2,4-pentanediols produces only the all-cis product, **7**, from the *meso*-diol. This is easily separated from the *dl*-diol derived racemic dioxane, **8**, by chromatography (SiO_2, C_6H_{14}) to obtain both isomers in pure form in yields of 29% and 57%, respectively. Similarly, (2*R*,4*R*)-(−)-2,4-pentanediol gave the interesting optically active acetal (+)-**8** (78%, $[\alpha]^{26}_D$ = +29.6° (neat)) (Figure 1).



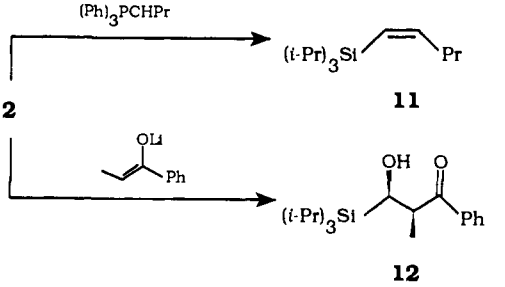
(2)

The reduction of **2** is easily accomplished with borane/dimethyl sulfide complex (BMS) (1:1) in THF (1 h, room temperature) to afford pure $TIPSCH_2OH$ (**9**) in 75% yield. Virtually quantitative conversion to **9** (≥95%) was observed by GC with BMS, $LiAlH_4$, and $NaBH_4$ as well as with EtMgBr and *n*-BuMgBr. By contrast, Li(*n*-Bu) gives the expected addition product **10a** (R = *n*-Bu, 78% (100% GC yield)). LiMe produces **10b** (R = Me, 78% (84% GC yield) more efficiently than does MeMgBr (65% GC yield). Grignard reagents lacking a β-hydride source also give **10** (**c**, R = Ph, 80%; **d**, R = C≡CPr, 74%).



(3)

To illustrate that **2** also undergoes the very highly stereoselective reactions which are common for bulky aldehydes, the Wittig olefination of **2** was examined under salt-free conditions,[15] which gave the *cis*-vinylsilane (**11**) (78%, 98% *Z*).[16] Also, the aldol reaction of **2** with the *Z* lithium enolate of propiophenone[17] produced the expected *syn*-aldol adduct (**12**) (65%, >97% syn).[18]



(4)

(13) Soderquist, J. A.; Miranda, E. I. *Tetrahedron Lett.* **1986**, *27*, 6305.
(14) Taylor, E. C.; Chiang, C. *Synthesis* **1977**, 467.
(15) Vedejs, E.; Meier, G. P.; Snoble, K. A. J. *J. Am. Chem. Soc.* **1981**, *103*, 2823. See also: Maryanoff, B. E.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863 and references cited therein.
(16) Ph_3PCHPr in PhMe[15] was less efficient (61%) and selective (c/t = 96:4 by capillary GC) perhaps due to trace amounts of Li-containing impurities. ^{13}C NMR ($CDCl_3$) *cis*-**11** δ 150.50, 123.14, 36.92, 22.97, 18.89, 14.02, 12.20 ppm. *trans*-**11** δ 149.33, 123.55, 39.51, 22.16, 18.65, 13.59, 12.36 ppm.
(17) (a) House, H. O.; Phillips, M. V.; Sayer, T. S. B.; Yan, C.-C. *J. Org. Chem.* **1978**, *43*, 700. (b) Heathcock, C. H.; Buse, C. T.; Kleschick, W. A.; Pirrung, M. C.; Sohn, J. E.; Lampe, J. *J. Org. Chem.* **1980**, *45*, 1066.

With these developments, formylsilanes emerge from their status as transient intermediates and laboratory curiosities to that of a rich new source of silicon-containing compounds.

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Supplementary Material Available: Listings of detailed procedures and complete spectral data for compounds **1–12** (14 pages). Ordering information is given on any current masthead page.

(18) For **12**, $^3J_{H(2)H(3)}$ = 1.3 Hz (δ 3.66, 4.19), which agrees well with the MMX-derived value for the syn (0.3 Hz) rather than the anti (12.8 Hz) isomer.[17b] Enolate to **2** addition at −78 °C gives a single aldol product (^{13}C NMR ($CDCl_3$) δ 206.6, 135.5, 133.2, 128.7, 128.3, 64.1, 42.0, 19.0, 18.98, 13.4, 11.1 ppm), whereas the reverse addition gives minor amounts of the anti isomer (δ 206.2, 43.7, 66.0, 13.5, 11.2 ppm) as well as recovered PhCOEt.

A de Novo Designed Protein Shows a Thermally Induced Transition from a Native to a Molten Globule-like State

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The de novo design of peptides and proteins[1] with predetermined structures provides an important test of our understanding of the principles that govern protein stability and folding. Several designed peptides and proteins have been described,[2,3] but the design of a compact, globular protein that shows all the hallmarks of a native protein has not yet been reported; instead, many of the designed proteins appear to adopt folded states with loosely packed hydrophobic cores such as those found in molten globules or compact intermediates (CI).[1,4] In this communication we describe

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(1) DeGrado, W. F.; Raleigh, D. P.; Handel, T. M. *Curr. Opin. Struct. Biol.* **1991**, *1*, 984–993.
(2) (a) DeGrado, W. F.; Wasserman, Z. R.; Lear, J. D. *Science* **1989**, *243*, 622–628. (b) Ho, S. W.; DeGrado, W. F. *J. Am. Chem. Soc.* **1987**, *109*, 6751–6758. (c) Regan, L.; DeGrado, W. F. *Science* **1988**, *241*, 976–978. (d) Handel, T. M.; DeGrado, W. F. *J. Am. Chem. Soc.* **1990**, *112*, 6710–6711. (e) Osterhout, J. L.; Handel, T. M.; Na, G.; Toumadje, A.; Long, R. C.; Connolly, P. J.; Hoch, J. C.; Johnson, W. C.; Live, D.; DeGrado, W. F. *J. Am. Chem. Soc.* **1992**, *114*, 331–337.
(3) (a) Moser, R. M.; Thomas, B.; Gutte, B. *FEBS Lett.* **1983**, *157*, 247–251. (b) Richardson, J. S.; Richardson, D. C. *Trends Biochem. Sci.* **1989**, 304–309. (c) Morii, H.; Ichimura, K.; Uedaira, H. *Chem. Lett.* **1990**, 1987–1990. (d) Johnsson, K.; Allemann, R. K.; Benner, S. A. In *Molecular Mechanisms in Bioorganic Processes*; Bleasdale, C., Golding, B. T., Eds.; Royal Society of Chemistry: Cambridge, 1990; pp 166–187. (e) Goraj, K.; Renard, A.; Martial, J. A. *Protein Eng.* **1990**, *3*, 259–266. (f) Regan, L.; Clark, N. D. *Biochemistry* **1990**, *29*, 10878–10883. (g) Hahn, K. W.; Klis, W. A.; Stewart, J. M. *Science* **1990**, *248*, 1544–1547. (h) Kaumaya, P. T.; Berndt, K. D.; Heidorn, D. B.; Trewhella, J.; Kezdy, J. F.; Goldberg, E. *Biochemistry* **1990**, *29*, 13–23. (i) Hecht, M. H.; Richardson, J. S.; Richardson, D. C.; Ogden, R. C. *Science* **1990**, *249*, 884–891. (j) Klauser, S.; Gantner, D.; Salgam, P.; Gutte, B. *Biochem. Biophys. Res. Commun.* **1991**, *179*, 1212–1219. (k) Ghadiri, R. M.; Soares, C.; Choi, C. *J. Am. Chem. Soc.* **1992**, *114*, 825–831. (l) Chin, T.-M.; Berndt, K. D.; Yang, N.-C. *J. Am. Chem. Soc.* **1992**, *114*, 2279–2280. (m) Zhou, N. E.; Kay, C. M.; Hodges, R. S. *J. Biol. Chem.* **1992**, *267*, 264–267.

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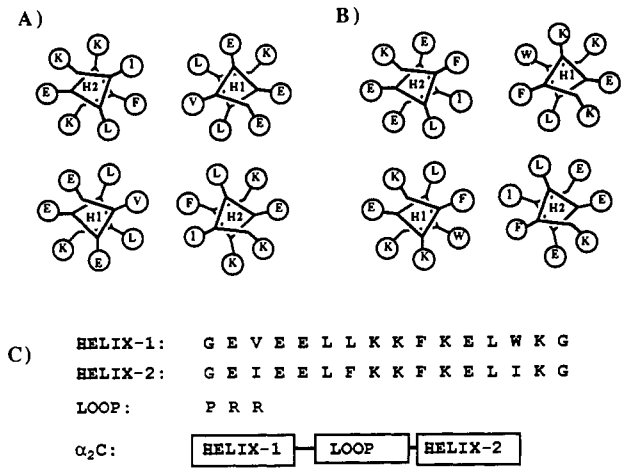

Figure 1. Helical wheel representation of α_2C. The packing of residues 2–8 of helix 1 with residues 15–9 of helix 2 is displayed in part A, and the packing of residues 9–15 of helix 1 with residues 8–2 of helix 2 is displayed in part B. The complete sequence is listed in part C. The N-terminus of the peptide is acetylated, and the C-terminus is amidated.

the first example of a de novo designed protein with most of the characteristics typical of native proteins including a well-dispersed NMR spectrum, a temperature-dependent near-UV circular dichroism (CD) spectrum, and a cooperative thermally-induced phase transition.

Recently, we described several four-helix bundles: α_1, which forms α-helical tetramers; α_2, which dimerizes to a four-helix bundle; and α_4, a single-chain four-helix bundle.[1,2] These species assumed extremely stable four-helix structures with physical properties intermediate between those of the native and CI states.[5] The hydrophobic core of these proteins, which consists exclusively of Leu, appears to be unusually flexible as compared to the native state. Presumably, the CI-like nature of α_4 arises because of the choice of Leu, which has a large number of broadly distributed rotomeric states.[6]

In order to design a more native-like interior, we replaced a number of the Leu residues in α_2 with more conformationally-constrained, β-branched and aromatic amino acids,[6] thereby also introducing shape complementarity into the helix–helix packing. We began by introducing a Val at position 3 and Ile at positions 22 and 33. To improve the packing, Phe residues were introduced at positions 10, 26, and 29, and a Trp was introduced at position 14 (Figure 1). The resulting peptide, α_2C, was synthesized by the solid-phase method.[7]

Size exclusion chromatography through Sephadex G50 indicates that the peptide elutes with an aggregation number of 2.1, consistent with dimer formation. In the idealized model of the peptide, the Trp side chain lies in a narrow groove between two of the helices and is partially exposed to solvent. At 278 K α_2C has a fluorescence emission maximum of 331 nm, consistent with partial exposure of the Trp side chain to solvent,[8] and the near-UV CD

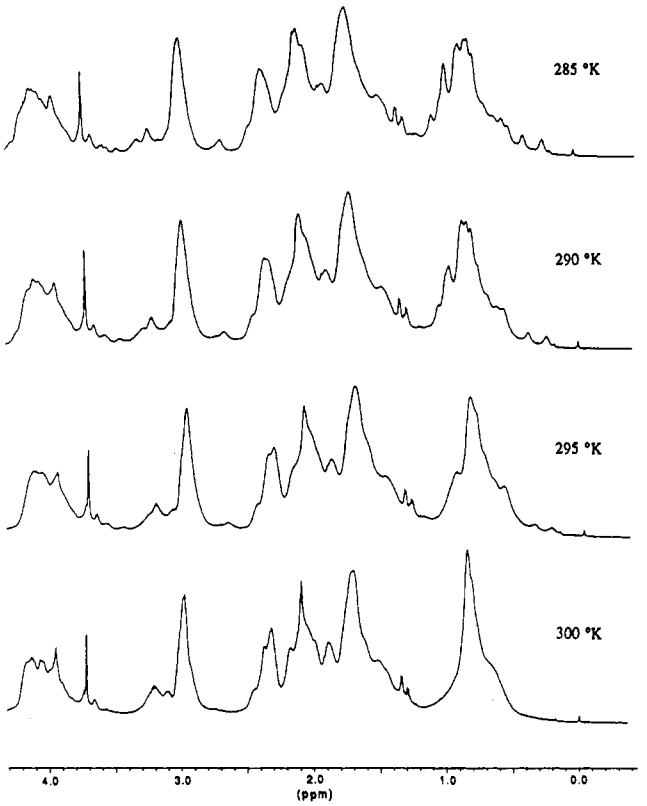

Figure 2. The upfield region of the NMR spectrum of α_2C as a function of temperature. The temperature is listed next to each spectrum. All spectra were recorded in D_2O at pH 7.0 on a Bruker AMX 600-MHz spectrometer using weak irradiation to saturate residual HOD. The peptide concentration was 1 mM, and the spectrum is independent of concentration over the range studied (80 μM–4 mM). Chemical shifts are given in parts per million from TMS.

spectrum shows several weak bands at this temperature. At 313 K the CD bands are lost, indicating that the aromatic side chains have undergone a transition from an asymmetric to a more averaged environment. In contrast, the helical content of the peptide is almost independent of temperature over the range 273–308 K, as judged by the mean residue ellipticity at 222 nm, which varies by less than 12%.

Below room temperature, the NMR spectrum is reminiscent of a folded, tightly packed protein, while above room temperature, the spectrum resembles that typically observed for molten globules (Figure 2). At low temperature, the spectrum is well dispersed and a number of ring-current-shifted methyl resonances are visible between 0 and 1.3 ppm. As the temperature is raised, these resonances decrease in intensity, and above 298 K, they all fall within a broad envelope centered near the random coil value. Parallel changes are observed in the aromatic region. Van't Hoff analysis of the NMR data, assuming a two-state transition, yields an enthalpy of 60 ± 20 kcal mol^{-1}. Although the observed transition may be more complicated than a simple two-state model would imply, the calculated value of ΔH is in reasonable agreement with the values reported for the unfolding transition of natural proteins.[9]

These results clearly demonstrate that α_2C has many of the characteristics of native proteins such as α-lactalbumin, including a cooperative thermal transition between a native-like state at low temperatures and a molten globule-like state at higher temperatures.[4] It is interesting to note that α_2C assembles into a protein with a structure approximately as complex as a simple protein such as intestinal calcium-binding protein, which has a C_2-symmetric four-helix structure arising from gene duplication of a two-helix motif.[10] α_2C retains two properties that are not entirely

(4) (a) Kuwajima, K.; Nitta, K.; Yoneyama, M.; Sugai, S. *J. Mol. Biol.* **1976**, *106*, 359–373. (b) Dolgikh, D. A.; Gilmanshin, R. A.; Brazhikov, E. V.; Bychkova, V. E.; Semisotnov, V. S.; Venyaminov, S. Yu.; Ptitsyn, O. B. *FEBS Lett.* **1981**, *136*, 311–315. (c) Ohgushi, M.; Wada, A. *FEBS Lett.* **1983**, *164*, 21–24. (d) Kuwajima, K. *Proteins* **1989**, *6*, 87–103. (e) Baum, J.; Dobson, C. M.; Evans, P. A.; Hanley, C. *Biochemistry* **1989**, *28*, 7–13. (f) Ptitsyn, O. B.; Pain, R. H.; Semisotnov, G. V.; Zerovnik, E.; Razgulyaer, O. L. *FEBS Lett.* **1990**, *262*, 20–24. (g) Kim, P. S.; Baldwin, R. L. *Annu. Rev. Biochem.* **1990**, *59*, 631–660. (h) Ptitsyn, O. B. *FEBS Lett.* **1991**, *285*, 176–181.

(5) Handel, T. M.; Williams, S.; DeGrado, W. F. Submitted.

(6) McGregor, M. J.; Islam, S. A.; Sternberg, M. J. E. *J. Mol. Biol.* **1987**, *198*, 775–791.

(7) The peptide was synthesized using a Milligen Model 9050 peptide synthesizer and purified to greater than 98% with reverse-phase HPLC. Amino acid analysis confirmed the expected composition, and fast atom bombardment mass spectrometry gave the correct $(M + H)^+$ of 4275.1.

(8) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983.

(9) Alexander, P.; Fahnestock, S.; Lee, T.; Orban, J.; Bryan, P. *Biochemistry* **1992**, *31*, 3597–3603.

consistent with the native state: (1) it binds δ-anilino-1-naphthalenesulfonate with a dissociation constant of approximately 50 μM; (2) the resonances in the proton NMR spectrum are somewhat broader than expected for a protein of this molecular weight, suggesting some mobility or aggregation. These results are not surprising, since only one of the helix/helix interfaces has been optimized. We are therefore working on further optimizing the packing of α_2C.

Acknowledgment. We thank Sharon Jackson and Arlene Rockwell for assistance in peptide synthesis and Tracy Handel for helpful discussions.

Supplementary Material Available: Fast atom bombardment mass spectrum of α_2C and plots of the intensity of the resolved methyl resonances in the NMR spectrum of α_2C as a function of temperature and of the intensity of the far-UV CD signal at 222 nm as a function of temperature (3 pages). Ordering information is given on any current masthead page.

(10) (a) Szebenyi, D. M. E.; Moffat, K. *J. Biol. Chem.* **1986**, *261*, 8761-8777. (b) Kretsinger, R. H. *Cold Spring Harbor Symp. Quant. Biol.* **1987**, *52*, 411.

Total Synthesis of Kuanoniamines and Dercitins

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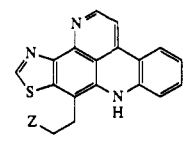
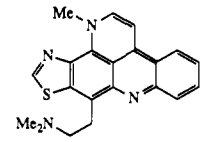
Kuanoniamines B–D (**1–3**)[2] and dercitins (**4**, **5**)[3] are structurally unique, highly cytotoxic thiazolopyridoacridine alkaloids obtained from marine sources (Scheme I).[4] Interestingly, the moderate potency observed for kuanoniamines is greatly enhanced in **5**, which exhibits not only strong antitumor activity in vitro and in vivo but also immunosuppressive and antiviral properties.[5] It should be noted that materials structurally related to **1–5** are known to be inhibitors of reverse transcriptase,[6] raising the possibility that kuanoniamines and dercitins may be active against HIV. Indeed, a recent report provides some support for this hypothesis.[7]

Scheme I



1 Z = *i*-BuCONH
2 Z = EtCONH
3 Z = MeCONH
4 Z = NMe_2



5

The new alkaloids are very rare substances, and in any event their compact aromatic framework does not lend itself to modification for the purpose of SAR studies. No synthetic approaches to this class of alkaloids are known.[8] Furthermore, the structure of **5** was originally misassigned and later corrected.[3] These problems conspire to seriously complicate any further investigation of the potentially important biological properties of **1–5**. In light of these facts, we launched a synthetic program with the intent of solving such problems. This effort has now culminated with the first total synthesis of **3–5**, as described below.

Construction of the ring system of **1–5** relied on the application of our pyridine-forming reaction as a key step.[9] Thus, ytterbium(III)-mediated cycloaddition of ethyl vinyl ether to enone **6** and treatment of the intermediate adduct with $HONH_2 \cdot HCl$ in MeCN at reflux furnished the pyridine **7**, which was converted to ketone **8** (Scheme II).[10] It was anticipated that the thiazole unit would be most readily installed at the stage of **8**. Indeed, bromination of the α-position of the carbonyl group (pyridinium tribromide)[11] and Traumann reaction[13] of crude **9**[12] furnished the expected aminothiazole **10**, which was efficiently deaminated[14] to the desired **11**.[15] Cleavage of the acetate gave alcohol **12**, from which mesylate **13** was obtained quantitatively. The routes to dercitins and kuanoniamines diverged at this point.

Kuanoniamine D (**3**), an especially active member of the omonimous family, was selected as our primary target. Thus, the mesylate **13** was advanced to amide **16** (Scheme III), from which totally synthetic **3**[15] was secured in a single step and in 62% chromatographed yield by triplet-sensitized photolysis (acetophenone, 150-W Sylvania sunlamp, Pyrex)[16] of the aromatic azide. This reaction proceeded with in situ oxidation of the primary photoproduct **17**, presumably through H-atom transfer to photoexcited acetophenone. The overall yield of **3**[17] from **6** was 10.0% over 12 steps.

(1) Recipient of the Robert A. Welch Predoctoral Fellowship.

(2) Carroll, A. R.; Scheuer, P. J. *J. Org. Chem.* **1990**, *55*, 4426.

(3) Dercitin: (a) Gunawardana, G. P.; Kohmoto, S.; Gunasekara, S. P.; McConnell, O. J.; Koehn, F. E. *J. Am. Chem. Soc.* **1988**, *110*, 4856. Norcitin: (b) Gunawardana, G. P.; Komoto, S.; Burres, N. S. *Tetrahedron Lett.* **1989**, *30*, 4359. (c) Gunawardana, G. P.; Koehn, F. E.; Lee, A. Y.; Clardy, J.; He, H.-Y.; Faulkner, J. D. *J. Org. Chem.* **1992**, *57*, 1523 (revised structures).

(4) Related alkaloids. Cystodytins: Kobayashi, J. I.; Cheng, J.-F.; Wälchli, M. R.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Ohizumi, Y. *J. Org. Chem.* **1988**, *53*, 1800, and ref 10. Diplamine: Charyulu, G. A.; McKee, T. C.; Ireland, C. M. *Tetrahedron Lett.* **1989**, *30*, 4201. Veramine: Molinski, T. F.; Ireland, C. M. *J. Org. Chem.* **1989**, *54*, 4256. Shermilamines: Carroll, A. R.; Cooray, N. M.; Poiner, A.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 4231. Segolines: Rudi, A.; Kashman, Y. *J. Org. Chem.* **1989**, *54*, 5331. Ascidemnin: Kobayashi, J. I.; Cheng, J. F.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1988**, *29*, 1177. Leptoclinidinones: DeGuzman, F. S.; Schmitz, F. J. *Tetrahedron Lett.* **1989**, *30*, 1069. Plakinidines: West, R. R.; Mayne, C. L.; Ireland, C. M.; Brinen, L. S.; Clardy, J. *Tetrahedron Lett.* **1990**, *31*, 3271. Amphimedin: Schmitz, F. J.; Agarwal, S. K.; Gunasekara, S. P.; Schmidt, P. G.; Schoolery, J. N. *J. Am. Chem. Soc.* **1983**, *105*, 4835. For an excellent review, see: Kobayashi, J.-I.; Ishibashi, M. In *The Alkaloids*; Brossi, A., Cordell, G. A., Eds.; Academic Press: San Diego, CA, 1992; Vol. 41, Chapter 2.

(5) Kuanoniamine D, a particularly active member of the family, shows an IC_{50} value against KB cells equal to 1.0 μg/mL (ref 2). Reported data against P388 leukemia for dercitin are as follows: IC_{50} = 50 ng/mL; T/C = 170% at 5 mg/kg. Immunosuppressant activity: 0% murine MLR at 10 ng/mL. Antiviral activity: strong inhibition of Herpes simplex I at 5 μg/well with moderate cytotoxicity; complete inhibition of murine A59 coronavirus at 1 μg/well with no cytotoxicity (ref 3).

(6) Inouye, Y.; Take, Y.; Oogose, K.; Kubo, A.; Nakamura, S. *J. Antibiot.* **1987**, *40*, 105.

(7) Taraporewala, I. B.; Cessac, J. W.; Chanh, T. C.; Delgado, A. V.; Schinazi, R. F. *J. Med. Chem.* **1992**, *35*, 2477.

(8) Preparation of thiazolo[5,4*a*]acridine substructures related to **1–5**: Barbe, J.; Boyer, G.; Carignano, I.; Elguero, J.; Galy, J.-P.; Morel, S.; Oughedani, R. *Tetrahedron Lett.* **1991**, *32*, 6709.

(9) Ciufolini, M. A.; Byrne, N. E. *J. Chem. Soc., Chem. Commun.* **1988**, 1230.

(10) Ciufolini, M. A.; Byrne, N. E. *J. Am. Chem. Soc.* **1991**, *113*, 8016. Compounds **8–16** and **18–19** emerged as 1:1 mixtures of diastereomeric rotamers as a result of axial dissymetry caused by restricted rotation of the azidophenyl group.

(11) Cf. Kornfeld, E. C.; Fornefeld, E. J.; Kline, B.; Mann, M. J.; Morrison, D. E.; Jones, R. G.; Woodward, R. B. *J. Am. Chem. Soc.* **1956**, *78*, 3087.

(12) This material is difficult to purify because of its propensity to undergo aromatization (-HBr).

(13) Traumann, V. *Liebigs Ann. Chem.* **1888**, *249*, 31.

(14) Cf. Doyle, M. P.; Dellaria, J. F., Jr.; Siegfrid, B.; Bishop, S. W. *J. Org. Chem.* **1977**, *42*, 3494.

(15) Melting points of selected compounds (uncorrected): **11**, mp 163–164 °C; synthetic **3**, yellow microcrystals changing to red-violet in acidic medium, decomposed at 260 °C without melting, lit.[2] mp >300 °C; synthetic **4**, yellow microcrystals changing to red-violet in acidic medium, mp 177–179 °C, lit.[3] mp 176 °C; **19**, 167–168 °C; **20**, 171–172 °C; **21**, 170–171 °C; synthetic **5**, purple microcrystals changing to red in acidic medium, mp 165–167 °C, lit.[3] mp 168 °C.

(16) (a) Lindley, J. M.; McRobbie, I. M.; Meth-Cohn, O.; Suschitzsky, H. *Tetrahedron Lett.* **1976**, *17*, 4513. (b) Lindley, J. M.; McRobbie, I. M.; Meth-Cohn, O.; Suschitzsky, H. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2194. See also ref 10.

(17) The spectral data for this synthetic material, including HRMS measurements, were in complete agreement with the literature. Unfortunately, we were unable to obtain an authentic sample of the natural product for the purpose of direct comparison.

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