d,l- and meso-Isochrysohermidin: Total Synthesis and Interstrand DNA Cross-Linking

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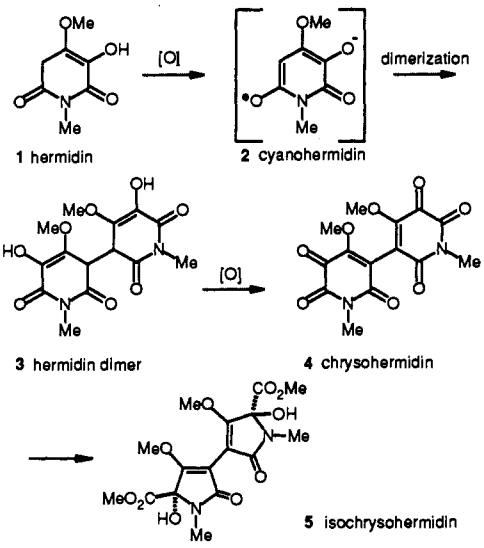
Abstract: A concise total synthesis of isochrysohermidin (**5**) is detailed based on a novel application of the *sym*-tetrazine → 1,2-diazine → pyrrole Diels–Alder strategy. Two consecutive $LUMO_{diene}$-controlled Diels–Alder reactions of 1,1,4,4-tetramethoxy-1,3-butadiene (**6**) with 3,6-bis(methoxycarbonyl)-1,2,4,5-tetrazine (**7**) followed by a double reductive ring contraction reaction of the symmetrical 4,4′-bi-1,2-diazine **8** provided **9** in a two-step reaction sequence that constitutes an effective new and general approach to functionalized 3,3′-bipyrroles. Following an efficient three-step reaction sequence for differentiation of the C2/C2′ and C5/C5′ methoxycarbonyl groups, a regioselective oxidative decarboxylation reaction of the cyclic endoperoxides derived from singlet oxygen [4 + 2] cycloaddition to each pyrrole of **12** provided *d,l*- and *meso*-isochrysohermidin (**5**). In agreement with expectations, both *d,l*- and *meso*-**5** proved to be effective interstrand DNA cross-linking agents, and the preliminary characterization of their interaction with duplex DNA is described.

Autoxidation of the colorless chromogen hermidin (**1**)[1] isolated from *Mercurialis perennis* L.[2] sequentially provides the transient blue radical anion cyanohermidin (**2**),[3] hermidin dimer (**3**),[1] and chrysohermidin (**4**)[1,4] and may precede rearrangement to isochrysohermidin (**5**),[5] a 2-oxo-3-pyrroline dimer first isolated from *Mercurialis leiocarpa* and recently shown to be related to **4** through a sodium methoxide-promoted rearrangement[5c] (Scheme I). This latter work also led to the demonstration that both *d,l*-**5**,[5a] a racemate, and *meso*-**5**[5b] are present in the samples derived from natural sources, and the *d,l*-diastereomer was unambiguously identified in X-ray crystallographic studies.[5a] Our examination of the unusual structure of **5** containing two symmetrical carbinolamides positioned orthogonally at the end of the 3,3′-bis-2-oxo-3-pyrroline skeleton suggested it may represent an ideal candidate for interstrand DNA cross-linking through reversible acetal exchange with duplex DNA nucleophilic sites. This interest, the unique structure of **5** and the current lack of naturally derived material, and the intriguing prospect that **5** may be biogenetically derived from $^1O_2$ addition to an appropriate 3,3′-bipyrrole precursor provided the incentive for the studies detailed herein.

Herein we provide full details[6] of a concise total synthesis of isochrysohermidin (**5**) based on the implementation of two consecutive heteroaromatic azadiene Diels–Alder reactions[7] of 1,1,4,4-tetramethoxy-1,3-butadiene with 3,6-bis(methoxycarbonyl)-1,2,4,5-tetrazine[8] (**6** + **7** → **8**) and a subsequent double reductive ring contraction reaction[9,10] of the 4,4′-bi-1,2-diazine **8** (**8** → **9**) representing a new and general approach to such 3,3′-bipyrroles and their derivatives. Consistent with the prospect

Scheme I



that **5** may be derived from an appropriately substituted 3,3′-bipyrrole, a final [4 + 2] $^1O_2$ addition to **12** followed by regioselective oxidative decarboxylation with endoperoxide fragmentation provided isochrysohermidin (**5**) as a readily separable mixture of stable *d,l*- and *meso*-diastereomers. In agreement with expectations, both *d,l*- and *meso*-**5** proved to be effective interstrand DNA cross-linking agents, and the preliminary characterization of their interaction with duplex DNA is detailed.

Total Synthesis of *d,l*- and *meso*-Isochrysohermidin. Double Diels–Alder reaction of **7** with 1,1,4,4-tetramethoxy-1,3-buta-

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(1) Swan, G. A. *Experientia* **1984**, *40*, 687.
(2) Haas, P.; Hill, T. G. *Biochem. J.* **1925**, *19*, 233; **1925**, *19*, 236.
(3) Forrester, A. R. *Experientia* **1984**, *40*, 688.
(4) Swan, G. A. *J. Chem. Soc., Perkin Trans. 1* **1985**, 1757.
(5) (a) Masui, Y.; Kawabe, C.; Mastumoto, K.; Abe, K.; Miwa, T. *Phytochemistry* **1986**, *25*, 1470. (b) Abe, K.; Okada, T.; Masui, Y.; Miwa, T. *Chem. Express* **1990**, *5*, 13. (c) Abe, K.; Okada, T.; Masui, Y.; Miwa, T. *Phytochemistry* **1989**, *28*, 960.
(6) Wasserman, H. H.; DeSimone, R. W.; Boger, D. L.; Baldino, C. M. *J. Am. Chem. Soc.* **1993**, *115*, 8457.
(7) Boger, D. L.; Weinreb, S. M. *Hetero Diels-Alder Methodology in Organic Synthesis*; Academic Press: San Diego, CA, 1987. Boger, D. L. *Tetrahedron* **1983**, *39*, 2869. Boger, D. L. *Chem. Rev.* **1986**, *86*, 781. Boger, D. L.; Patel, M. In *Progress in Heterocyclic Chemistry*; Suschitzky, H., Scriven, E. F. V., Eds.; 1989; Vol. 1, p 30. Boger, D. L. *Bull. Soc. Chim. Belg.* **1990**, *99*, 599.
(8) Boger, D. L.; Panek, J. S.; Coleman, R. S.; Sauer, J.; Huber, F. X. *J. Org. Chem.* **1985**, *50*, 5377. Boger, D. L.; Panek, J. S.; Patel, M. *Org. Synth.* **1992**, *70*, 79. Spencer, G. H., Jr.; Cross, P. C.; Wiberg, K. B. *J. Chem. Phys.* **1961**, *35*, 1939. Sauer, J.; Mielert, A.; Lang, D.; Peter, D. *Chem. Ber.* **1965**, *98*, 1435. Curtius, Th.; Darapsky, A.; Muller, E. *Chem. Ber.* **1906**, *39*, 3410; **1907**, *40*, 84; **1908**, *41*, 3161. Curtius, Th.; Lang, J. *J. Prakt. Chem.* **1888**, *38*, 531. Hantzsch, A.; Lehmann, M. *Chem. Ber.* **1900**, *33*, 3668. Hantzsch, A.; Silberrad, O. *Chem. Ber.* **1900**, *33*, 58.
(9) Boger, D. L.; Coleman, R. S.; Panek, J. S.; Yohannes, D. *J. Org. Chem.* **1984**, *49*, 4405.
(10) Boger, D. L.; Patel, M. *J. Org. Chem.* **1988**, *53*, 1405. Boger, D. L.; Patel, M. *Tetrahedron Lett.* **1987**, *28*, 2499.

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Scheme II

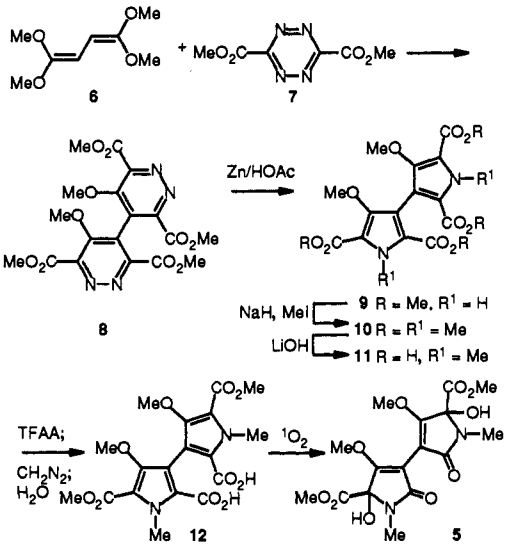


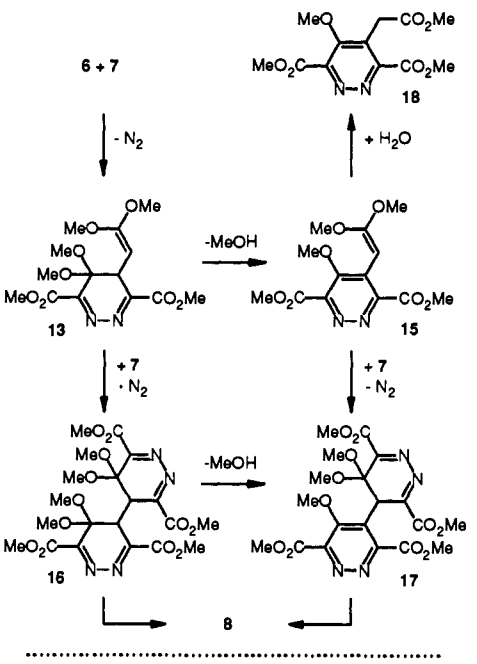
Table I. Representative Results of a Study of the Diels–Alder Reaction of **6** and **7**

entry	7 (equiv)	solvent	temp (°C)	time (h)	product (% yield)
1	1.0	CH_2Cl_2	22	0.3	**13** (95)[a]
2	1.0	C_6H_6	22	0.5	**13** (92)[a]
3	1.0	dioxane	22	8	**13** (95)[a,b]
4	1.0	$CHCl_3$	22	8	**18** (87)[b]
5	1.0	toluene	105	40	**18** (94)[b]
6	3.0	C_6H_6	80	72	**8** (25–35), **16** (15–25)[c]
7	4.0	dioxane	100	52	**8** (25), **16** (29)[c]
	4.0	dioxane	100	120	**8** (32), **16** (21)[c]
8	2.0	DMF	22	48	**8** (21)
9	3.0	C_6H_6	80	72	
		C_6H_6–HOAc	80	60	**8** (50–56)
10	2.0	$CHCl_3$–4-Å MS	22	48	**8** (23)
11	4.5	$CHCl_3$–4-Å MS	60	144	**8** (65)

[a] Spectroscopic (^{1}H NMR) determination; **18** was isolated after SiO_2 chromatography in 95% (entry 1) and 92% (entry 2) yield. [b] **18** was isolated after SiO_2 chromatography, and the prolonged reaction time at 22 °C did not result in a subsequent conversion to **8** or **16**. [c] **16** precipitates from the reaction mixture.

diene[11] (4.5 equiv of **7**, 60 °C, $CHCl_3$, 0.9 g of 4-Å molecular sieves/mmol, 144 h, 65%) provided **8** in good yield under selected reaction conditions (Scheme II). In a detailed study of the conversion to provide **8**, the initial reaction of **7** with the electron-rich dienophile **6** was found to proceed rapidly (<30 min) at room temperature and was accompanied by the vigorous evolution of nitrogen (Table I and Scheme III). Direct assay of the reaction mixture after 20 min (22 °C, CH_2Cl_2) provided **13**[12] (95%) and purification of the reaction product by SiO_2 chromatography afforded **18**[13] (94%) resulting from hydrolysis of **13**. The second [4 + 2] cycloaddition reaction of **6** with **13** or its methanol elimination product **15** proved substantially slower (80 °C, 72 h) and may be attributed to the increased steric hindrance or decreased nucleophilic character of the intermediate dienophiles

Scheme III



13 and **14**/**15**, respectively (Scheme III). Consistent with past studies, the aromatization steps requiring the loss of methanol from the intermediate 4,5- or 1,4-dihydro-1,2-diazines proved slow and occasionally problematic, leading to the isolation of **16**,[14] which would precipitate from a benzene reaction mixture. Contrary to expectations, the unaromatized cycloadducts **13** and **16** were isolated as the 4,5-dihydro versus 1,4-dihydro tautomeric structure (*i.e.*, **14**), and this was most evident from the ^{1}H and ^{13}C NMR[12,14] clear observation of the C4-CH signal. Conversion of **16** to the desired 4,4′-bi-1,2-diazine **8** could be accomplished by treatment with HOAc–C_6H_6[15,16] (80 °C, 48 h, 93%), and this provided **8** in much higher conversions than treatment with 4-Å molecular sieves,[17] PPA, *p*-TsOH–C_6H_6, K_2CO_3–DMF, pyridine, or potassium *tert*-butoxide. In practice, the overall conversion of **6** + **7** to **8** (50–56%) proved most effective if the initial [4 + 2] cycloaddition reactions were conducted in C_6H_6 to provide a mixture of **16**–**17** and **8** (22 °C, 20 min; 80 °C, 72 h, during which time **16** would precipitate from the reaction mixture) followed by the addition of HOAc (80 °C, 60 h) to catalyze the elimination of methanol to provide **8**. This latter procedure proved slightly less effective but more reproducible at providing **8** than that initially conducted in $CHCl_3$ in the presence of 4-Å molecular sieves. Presumably, the $CHCl_3$ reaction conditions benefit from the presence or generation of catalytic HCl in the reaction mixture, which, in conjunction with the 4-Å molecular sieves,[17] catalyzes the sluggish aromatization reaction.

1,2-Diazine reductive ring contraction effected by treatment of **8** with excess activated zinc in glacial acetic acid[9] (40 equiv of Zn, HOAc, 22 °C, 24 h, 68%) provided the 3,3′-bipyrrole **9** in exceptionally good yield provided freshly activated zinc[18] was employed. This unusual reaction which proceeds by zinc reduction to the corresponding 1,4-dihydro-1,2-diazine followed by slow reductive cleavage of the nitrogen–nitrogen bond and subsequent imine condensation[8] provided **9** in surprisingly high yield given

(11) Scheeren, J. W.; Aben, R. W. *Tetrahedron Lett.* **1974**, *12*, 1019.

(12) For **13**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.20 (d, 1H, *J* = 9.5 Hz, vinyl CH), 3.93 (s, 3H, CO_2Me), 3.91 (s, 3H, CO_2Me), 3.63 (s, 3H, vinyl OMe), 3.52 (s, 3H, vinyl OMe), 3.34 (s, 3H, OMe), 3.32 (s, 3H, OMe), 3.14 (d, 1H, *J* = 9.5 Hz, C5-H).

(13) For **18**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.09 (s, 2H, CH_2), 4.08 (s, 3H, OMe), 4.03 (s, 3H, CO_2Me), 3.99 (s, 3H, CO_2Me), 3.72 (s, 3H, aliphatic CO_2Me); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.6 (e, CO_2Me), 165.0 (e, CO_2-Me), 164.8 (e, CO_2Me), 157.2 (e, C4), 153.0 (e, C6), 147.8 (e, C5), 128.5 (e, C3), 62.1 (o, OMe), 53.5 (o, CO_2Me), 52.4 (o, CO_2Me), 53.3 (o, CO_2Me), 30.2 (e, CH_2); EIMS *m/e* 298 (M^+, 8), 267 (23), 225 (base), 197 (18), 181 (16), 180 (15), 121 (30), 59 (93); CIMS (2-methylpropane) *m/e* 299 (M + H^+, base).

(14) For **16**: mp 258–260 °C dec; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.99 (s, 3H, CO_2Me), 3.89 (s, 3H, CO_2Me), 3.41 (s, 1H, C4-CH), 3.29 (s, 3H, OMe), 3.09 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.0 (e, CO_2Me), 161.4 (e, CO_2Me), 156.0 (e, C3), 155.2 (e, C6), 95.7 (e, C5), 54.0 (o, CO_2Me), 53.4 (o, CO_2Me), 53.1 (o, OMe), 50.2 (o, OMe), 32.8 (o, C4); IR (KBr) ν_{max} 2956, 1742, 1440, 1356, 1272, 1235, 1111, 1085 cm^{-1}.

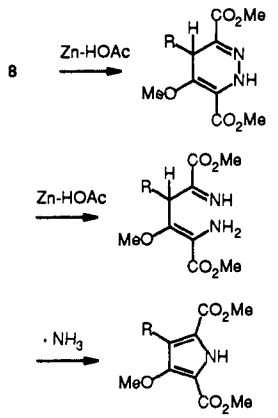
(15) Boger, D. L.; Zhang, M. *J. Am. Chem. Soc.* **1991**, *113*, 4230.

(16) Boger, D. L.; Dang, Q. *Tetrahedron* **1988**, *44*, 3379.

(17) Boger, D. L.; Panek, J. S.; Meier, M. M. *J. Org. Chem.* **1982**, *47*, 895.

(18) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: Oxford, U.K., 1988; p 360.

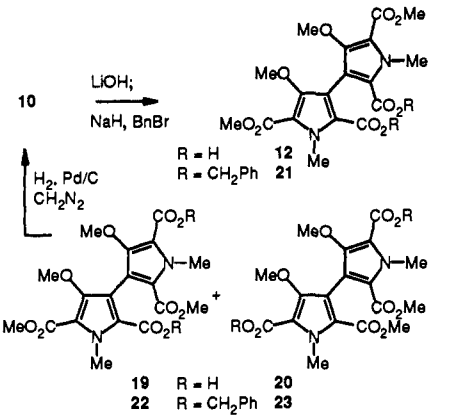
Scheme IV

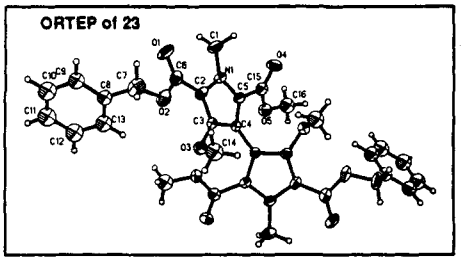


the potential competitive reactions of the intermediate reduction products (Scheme IV). Comparable results were obtained when the reduction was conducted in trifluoroacetic acid (47%) although this alternative[10] was not pursued in detail.

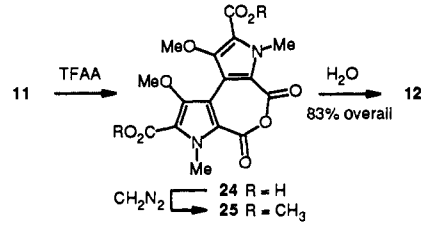
N-Methylation of **9** provided **10** uneventfully in DMF (NaH, CH_3I, 0–25 °C, 98%). A remaining selective differentiation of the C2/C2′ and C5/C5′ methoxycarbonyl groups was required to provide **12** penultimate to implementation of a directed oxidative decarboxylation reaction of the projected $^1O_2$ adduct with **12**.[19,20] Despite close literature precedent,[21,22] initial and extensive attempts to selectively differentiate the C2/C2′ and C5/C5′ methoxycarbonyl groups through ester hydrolysis under basic (1–2.5 equiv of LiOH, 22 °C, THF–CH_3OH–H_2O 3:2:1)[10,19] or acidic reaction conditions[22] provided little or no selectivity. Although clean monohydrolysis could be accomplished employing 1 equiv of LiOH (THF–CH_3OH–H_2O 3:2:1, 5 °C, 75 h), the reaction provided a 2:1 mixture of C2 (66%)[23] and C5 (31%)[24] monocarboxylic acids. Similarly, efforts to selectively esterify the tetraacid **11** under acidic or basic conditions failed to provide an effective differentiation of the C2/C2′ and C5/C5′ carboxylates. In fact, treatment of **11** with catalytic HCl–CH_3OH (0–22 °C, 2 h) led to clean decarboxylation to provide 4,4′-dimethoxy-1,1′-dimethyl-3,3′-bipyrrole[25] in surprisingly good conversion (93%). Nonetheless, a mixture of diacids **12** and **19–20** derived from controlled hydrolysis of **10** under basic conditions (2.0 equiv of LiOH, THF–CH_3OH–H_2O 3:1:1, 22 °C, 72 h) could be generated in initial studies and separated to provide samples of the authentic materials (Scheme V). Isolation and separation of the hydrolysis products were facilitated by subsequent conversion of the acidic reaction products to the corresponding benzyl esters (5.5 equiv of $PhCH_2Br$, 5.5 equiv of K_2CO_3, 0.1 equiv of *n*-Bu_4NI, DMF, 22 °C, 48 h). The symmetrical dibenzyl esters **21** (29%) and **23** (31%) as well as the unsymmetrical dibenzyl ester **22** (37%) were isolated along with a trace mixture of tribenzyl esters. Unambiguous assignment of the structures of the two symmetrical dibenzyl esters **21** versus **23** was made on the basis

Scheme V





Scheme VI



of the single-crystal X-ray structure determination of **23**.[26] Deprotection of **21** through catalytic transfer hydrogenolysis (HCO_2NH_4–10% Pd-C, THF–CH_3OH–H_2O, 100%) provided the desired diacid **12** while similar deprotection of **23** provided the isomeric symmetrical diacid **20**. Intermediates **22** and **23** as well as the trace mixture of tribenzyl esters could be combined, debenzylated, and recycled (CH_2N_2) to provide **10** in high yield (*ca.* 70%).

Although the efforts provided **12** suitable for further study and the recycling of **22** and **23** proved efficient, the generation of **12** by this approach proved cumbersome. A more effective differentiation of the C2/C2′ and C5/C5′ methoxycarbonyl groups with clean conversion to **12** was accomplished by exhaustive hydrolysis of **10** to provide the tetraacid **11** followed by selective cyclic anhydride formation to provide **24** (Scheme VI). Reesterification of the C5/C5′ carboxylic acids with diazomethane provided **25**, and subsequent addition of water to the cyclic anhydride provided **12** identical to the material previously prepared. Conversion of tetraacid **11** to the cyclic anhydride **24** proved uniquely successful upon treatment with trifluoroacetic anhydride.[27] A variety of alternative carboxylic acid activating reagents were examined for the preparation of **24** and proved to be ineffective, including DCC,[28a] EDCI, BOP-Cl,[28b] 2-mesitylenesulfonyl chloride,[28c] 2,4,6-$Cl_3C_6H_2COCl$,[28d] acetyl chloride,

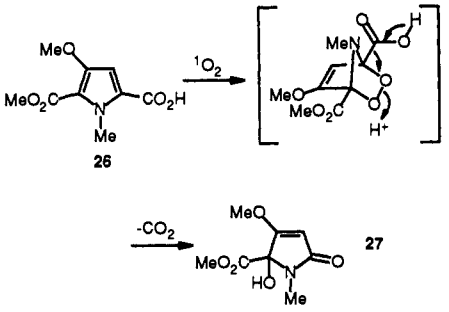
(19) Boger, D. L.; Baldino, C. M. *J. Org. Chem.* **1991**, *56*, 6942.
(20) Wasserman, H. H.; Frechette, R.; Rotello, V. M.; Schulte, G. *Tetrahedron Lett.* **1991**, *32*, 7571.
(21) Rapoport, H.; Bordner, J. *J. Org. Chem.* **1964**, *29*, 2727.
(22) Rapoport, H.; Willson, C. D. *J. Am. Chem. Soc.* **1962**, *84*, 630. Rapoport, H.; Holden, K. G. *J. Am. Chem. Soc.* **1962**, *84*, 635.
(23) ^1H NMR ($CDCl_3$, 200 MHz) δ 4.24 (s, 3H, NMe), 4.18 (s, 3H, NMe), 3.93 (s, 3H, OMe), 3.90 (s, 3H, OMe), 3.73 (s, 3H, CO_2Me), 3.68 (s, 3H, CO_2Me), 3.62 (s, 3H, CO_2Me).
(24) ^1H NMR ($CDCl_3$, 200 MHz) δ 4.23 (s, 3H, NMe), 4.18 (s, 3H, NMe), 3.92 (s, 3H, OMe), 3.91 (s, 3H, OMe), 3.73 (s, 3H, CO_2Me), 3.66 (s, 3H, CO_2Me), 3.63 (s, 3H, CO_2Me).
(25) For 4,4′-dimethoxy-1,1′-dimethyl-3,3′-bipyrrole: ^1H NMR ($CDCl_3$, 300 MHz) δ 6.81 (d, 1H, J = 2.5 Hz, C2), 6.12 (d, 1H, J = 2.5 Hz, C5), 3.74 (s, 3H, OMe); IR (KBr) ν_{max} 2926, 1542, 1515, 1458, 1444, 1388, 1340, 1265, 1156, 1049 cm^{-1}.
(26) The author has deposited the atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates may be obtained upon request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.
(27) Duckworth, A. C. *J. Org. Chem.* **1962**, *27*, 3146.

Table II. Representative Results of the Reaction of Singlet Oxygen with **26**

$^1O_2$ source	solvent	time (h)	temp (°C)	27
O_2, $h\nu^a$	*i*-PrOH–H_2O (3:1)	1	22	80%
O_2, $h\nu^a$	CH_3CN–H_2O (3:1)	1	22	92%
O_2, $h\nu^a$	CH_3CN–H_2O (3:1) pyridine (1.2 equiv)	1	22	72%
O_2, $h\nu^a$	CH_3CN–H_2O–pyridine (6:4:1)	1	22	63%
$(PhO)_3P$–O_3 (2.0 equiv)	CH_3CN–CH_2Cl_2	3	−78 to 22	48%
$(PhO)_3P$–O_3 (4.0 equiv)	*i*-PrOH–CH_2Cl_2	5	−78 to 22	23%

[a] Rose Bengal (8 mequiv), quartz immersion well, Hanovia high-pressure mercury lamp (450 W), uranium yellow glass filter (transmits > 330 nm), O_2, 22 °C.

Scheme VII



and acetic anhydride.[28e] Presumably this may be attributed to the enhanced reactivity of the trifluoroacetyl mixed anhydrides, the entropic assistance for closure of the half acid mixed anhydride to the cyclic anhydride, and the ease of subsequent hydrolysis of the terminal trifluoroacetyl mixed anhydrides by adventitous water present upon workup. Consistent with these suppositions, the treatment of **11** with acetic anhydride (0.20 M, 55 °C, 1 h) provided the linear tetraacetyl mixed anhydride (93%).[29] Although the conversion of **10** to **12** with differentiation of C2/C2′ and C5/C5′ methoxycarbonyl groups formally required four steps, its execution proved simple, efficient (83% overall), and capable of conduct without deliberate purification of the intermediates.

Double $^1O_2$ addition to the symmetrical 3,3′-bipyrrole **12** followed by in situ low-temperature fragmentation, a directed oxidative decarboxylation,[19] was anticipated to provide **5** as a mixture of *d,l*- and *meso*-diastereomers. This reaction was first examined in detail with **26** employing a number of $^1O_2$ sources and was found to provide predominantly or exclusively **27** derived from the $^1O_2$ [4 + 2] cycloaddition with the pyrrole followed by oxidative decarboxylation (Table II and Scheme VII).[19] Unlike the results generally obtained in the $^1O_2$ reaction with simple pyrroles,[30-32] the low-temperature oxidative decarboxylation reaction following $^1O_2$ [4 + 2] cycloaddition provided high yields of **27** directly under a variety of reaction conditions.[33,34,35,36,37]

Despite these preliminary studies, their extension to the conversion of **12** to **5** did not prove straightforward. The double addition of $^1O_2$ to **12** was accomplished best utilizing the photosensitized $^1O_2$ generation reaction conditions, and the conversion of **12** to **5** was observed *only* when the reaction was conducted in the presence of pyridine[20] or collidine (Table III). It is likely that the added base serves to deprotonate the carboxylic acid, thereby accelerating the oxidative decarboxylation fragmentation reaction following $^1O_2$ [4 + 2] cycloaddition, and that the use of a hindered base (*i.e.*, collidine versus pyridine) serves to minimize competitive side reactions including its direct addition to and cleavage of the cyclic endoperoxide. The reaction failed to provide **5** under a wide variety of alternative reaction conditions, and in a limited comparison, the use of triphenyl phosphite ozonide[33] as the source of $^1O_2$ generally provided isochrysohermidin in lower conversions. Early studies of the conversion of **12** to **5** were disappointing due to unanticipated problems that accompanied attempts to purify **5** by standard chromatography (SiO_2). Although the amount of *d,l*- and *meso*-**5** detected (TLC or ^1H NMR) in the crude reaction mixtures was low, it was subsequently determined that combining the materials with an R_f of 0.2–0.5 (SiO_2, EtOAc, 70–80% yield), which contained *d,l*-**5** (R_f 0.39) and *meso*-**5** (R_f 0.30), their ring opened isomers, and potentially their isomeric six-membered carbinolamides,

(28) (a) Brown, D.; Stevenson, R. *Tetrahedron Lett.* **1964**, 3213. (b) Cabre-Castellvi, J.; Palomo-Coll, A.; Palomo-Coll, A. L. *Synthesis* **1981**, 616. (c) Gerlach, H.; Oertle, K.; Thalmann, A.; Servi, S. *Helv. Chim. Acta* **1975**, *58*, 2036. (d) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (e) Rapoport, H.; Lee, D. L.; Morrow, C. J. *J. Org. Chem.* **1974**, *39*, 893.

(29) ^1H NMR ($CDCl_3$, 300 MHz) δ 4.25 (s, 3H, NMe), 3.64 (s, 3H, OMe), 2.29 (s, 3H, COMe), 1.93 (s, 3H, COMe); IR (KBr) ν_{max} 1812, 1773, 1735, 1521, 1400, 1370, 1140, 1048, 1000, 971 cm^{-1}.

(30) Lightner, D. A.; Bisacchi, G. S.; Norris, R. D. *J. Am. Chem. Soc.* **1976**, *98*, 802.

(31) Lightner, D. A.; Quistad, G. B. *J. Heterocycl. Chem.* **1973**, *10*, 273.

(32) Lightner, D. A.; Crandall, D. C. *Experientia* **1973**, *29*, 262.

(33) Foote, C. S.; Wexler, S.; Ando, W.; Higgins, R. *J. Am. Chem. Soc.* **1968**, *90*, 975. Foote, C. S.; Wexler, S.; Ando, W. *Tetrahedron Lett.* **1965**, 4111.

(34) Wasserman, H. H.; Scheffer, J. R.; Cooper, J. L. *J. Am. Chem. Soc.* **1972**, *94*, 4991.

(35) Sinden, R. R.; Hoepfner, R. W.; Ussery, D. W. *Meth. Enzymol.* **1992**, *212*, 242. Hopkins, P. B.; Millard, J. T.; Woo, J.; Weidner, M. F.; Kirchner, J. J.; Sigurdsson, S. T.; Raucher, S. *Tetrahedron* **1991**, *47*, 1415.

(36) For representative naturally occurring interstrand DNA cross-linking agents, see the following. (a) Psoralen: Tomic, M. T.; Wemmer, D. E.; Kim, S. H. *Science* **1987**, *238*, 1722. Yeung, A. T.; Jones, B. K.; Chu, C. T. *Biochemistry* **1988**, *27*, 3204. Miller, P. S.; Bahn, P. *Bioconjugate Chem.* **1990**, *1*, 82. Gia, O.; Magno, S. M.; Garbes, A.; Colonna, F. P.; Palumbo, M. *Biochemistry* **1992**, *31*, 11818. (b) Mitomycin: Iyer, V. N.; Szybalski, W. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *50*, 355. Iyer, V. N.; Szybalski, W. *Science* **1964**, *145*, 55. Tomasz, M.; Mercado, C. M.; Olson, J.; Chatterjie, N. *Biochemistry* **1974**, *13*, 4878. Zein, N.; Kohn, H. *J. Am. Chem. Soc.* **1986**, *108*, 296. Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K. *Science* **1987**, *235*, 1204. Teng, S. P.; Woodson, S. A.; Crothers, D. M. *Biochemistry* **1989**, *28*, 3901. Kumar, S.; Lipman, R.; Tomasz, M. *Biochemistry* **1992**, *31*, 1399. Li, V. S.; Kohn, H. *J. Am. Chem. Soc.* **1991**, *113*, 275. (c) FR900482 and FR66979: Williams, R. M.; Rajski, S. R. *Tetrahedron Lett.* **1992**, *33*, 2929. Woo, J.; Sigurdsson, S. Th.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 1199. (d) Pyrrolizidine alkaloids: White, I. N. H.; Mattocks, A. R. *Biochem. J.* **1972**, *128*, 291. Petry, T. W.; Bowden, G. T.; Huxtable, R. J.; Sipes, I. G. *Cancer Res.* **1984**, *44*, 1505. Reed, R. L.; Ahern, K. G.; Pearson, G. D.; Buhler, D. R. *Carcinogenesis* **1988**, *9*, 1355. Woo, J.; Sigurdsson, S. Th.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 3407. (e) Carzinophilin (azinomycin B): Terawaki, A.; Greenberg, J. *Biochem. Biophys. Acta* **1966**, *119*, 59. Terawaki, A.; Greenberg, J. *Nature* **1966**, *209*, 481. Lown, J. W.; Begleiter, A.; Johnson, D.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 110. Armstrong, R. W.; Salvati, M. E.; Nguyen, M. *J. Am. Chem. Soc.* **1992**, *114*, 3144.

(37) For representative synthetic interstrand DNA cross-linking agents, see the following. (a) Nitrogen mustards: Brooks, P.; Lawley, P. D. *J. Chem. Soc.* **1961**, 3923. Geiduschek, E. P. *Proc. Natl. Acad. Sci. U.S.A.* **1961**, *47*, 950. Brooks, P.; Lawley, P. D. *Biochem. J.* **1961**, *80*, 496. Rink, S. M.; Solomon, M. S.; Taylor, M. J.; Rajur, S. B.; McLaughlin, L. W.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 2551. Millard, J. T.; Raucher, S.; Hopkins, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 2459. (b) Diepoxybutane: Millard, J. T.; White, M. M. *Biochemistry* **1993**, *32*, 2120. (c) U-77,779: Mitchell, M. A.; Kelly, R. C.; Wicnienski, N. A.; Hatznenbuhler, N. T.; Williams, M. G.; Petzold, G. L.; Slightom, J. L.; Siemieniak, D. R. *J. Am. Chem. Soc.* **1991**, *113*, 8994. Lee, C. S.; Gibson, N. W. *Biochemistry* **1993**, *32*, 2592. (d) C_2BI–$CDPI_2$: Reference 40. (e) *cis*-Platin: Erickson, L. C.; Zwelling, L. A.; Ducore, J. M.; Sharkey, N. A.; Kohn, K. W. *Cancer Res.* **1981**, *41*, 2791. Brouwer, J.; Fichtinger-Schepman, A. M. J.; van de Putte, P.; Reedijk, J. *Cancer Res.* **1982**, *42*, 2416. Leng, M.; Rahmouni, A. *Biochemistry* **1987**, *26*, 7229. Eastman, A. *Biochemistry* **1985**, *24*, 5027. Szymkowski, D. E.; Yarema, K.; Essigmann, J. M.; Lippard, S. J.; Wood, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 10772. Cullinane, C.; Wickham, G.; McFadyen, W. D.; Denny, W. A.; Palmer, B. D.; Phillips, D. R. *Nucleic Acids Res.* **1993**, *21*, 393. Bradley, L. J. N.; Yarema, K. J.; Lippard, S. J.; Essigmann, J. M. *Biochemistry* **1993**, *32*, 982. (f) Dimeric anthramycin analogs: Farmer, J. D., Jr.; Rudnicki, S. M.; Suggs, J. W. *Tetrahedron Lett.* **1988**, *29*, 5105. Bose, D. S.; Thompson, A. S.; Ching, J.; Hartley, J. A.; Berardini, M. D.; Jenkins, T. C.; Neidle, S.; Hurley, L. H.; Thurston, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 4939. (g) Diaziridinylbenzoquinones: Lee, C. S.; Hartley, J. A.; Berardini, M. D.; Butler, J.; Siegel, D.; Ross, D.; Gibson, N. W. *Biochemistry* **1992**, *31*, 3019. Friedlos, F.; Quinn, J.; Knox, R. J.; Roberts, J. J. *Biochem. Pharmacol.* **1992**, *43*, 1249. Berardini, M. D.; Souhami, R. L.; Lee, C. S.; Gibson, N. W.; Butler, J.; Hartley, J. A. *Biochemistry* **1993**, *32*, 3306. (h) Nitrous acid: Kirchner, J. J.; Hopkins, P. B. *J. Am. Chem. Soc.* **1991**, *113*, 4682. Kirchner, J. J.; Sigurdsson, S. Th.; Hopkins, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 4021. (i) Formaldehyde: Huang, H.; Solomon, M. S.; Hopkins, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 9240.

Table III. Singlet Oxygen Reaction of **12**[a]

solvent	*d,l*-**5** (%)
i-PrOH–H_2O (3:1)	0
CH_3CN–H_2O (3:1)	0
i-PrOH–pyridine–H_2O (3:1:1)	6[b]
	16[c]
CH_3CN–pyridine–H_2O (3:1:1)	5[b]
i-PrOH–H_2O–DBU (6:2:1)	0
pyridine–H_2O (1:1)	10[b]
	23[c]
pyridine–H_2O (1:3)	28[c]
pyridine–H_2O (3:1)	11[c]
collidine–H_2O (1:10)	26[c]
collidine–H_2O (1:3)	40[c]
collidine–H_2O (1:1)	36[c]

[a] $^1O_2$ source: Rose Bengal (8 mequiv), Hanovia high-pressure lamp (450 W), uranium yellow glass filter (transmits > 330 nm), O_2, 22 °C, 1 h. [b] Product (R_f 0.39, SiO_2, EtOAc) isolated by chromatography (SiO_2, EtOAc) and recrystallized (EtOAc) to provide pure *d,l*-**5**. [c] Product (R_f 0.2–0.5) isolated by chromatography (SiO_2, EtOAc) and recrystallized (EtOAc) to provide pure *d,l*-**5**. *meso*-**5** and isomers of **5** remained in the mother liquor.

Table IV. ^1H NMR (400 MHz) of Isochrysohermidin (**5**)[a]

assignment	$CDCl_3$	acetone-d_6	CD_3OD	DMSO-d_6	pyridine-d_5	CF_3CO_2D
			d,l-**5**			
OH	6.62	6.32		7.55		
OCH_3	3.98	3.95	3.98	3.84	4.14	4.02
CO_2CH_3	3.83	3.77	3.78	3.68	3.73	3.88
NCH_3	2.84	2.73	2.78	2.63	3.01	2.87
			meso-**5**			
OH	4.65	6.20		7.45		
OCH_3	3.98	3.94	3.97	3.83	4.20	4.01
CO_2CH_3	3.87	3.78	3.80	3.70	3.68	3.90
NCH_3	2.78	2.71	2.77	2.63	3.06	2.87

[a] ^1H NMR of separated and pure samples of *d,l*- and *meso*-**5**.

followed by selective crystallization of *d,l*-**5** from the mixture provided **5** in much higher conversion. Using this procedure, pure *d,l*-**5** was isolated in 37–43% overall yield by direct crystallization from EtOAc, and subsequent repeated chromatography of the remaining mother liquors provided an additional 8% of *meso*-**5**. Under the conditions of the crystallization, *d,l*-**5** and *meso*-**5** do not appear to interconvert and the isolation of pure *meso*-**5** by repeated chromatography was accompanied by a loss of product. Our observations suggest that the $^1O_2$ addition–fragmentation proceeds in conversions of *ca.* 70–80% although we have not successfully isolated pure *d,l*- and *meso*-**5** in these conversions.

In the initial stage of our studies, synthetic **5** was isolated as a pure 1:1 mixture of *meso*- and *d,l*-diastereomers by repeated chromatography. The two diastereomers were further separated by selective crystallization to provide pure *d,l*-**5** while trituration of the solid mixture of the two diastereomers (1:1) with ethyl acetate selectively solubilized pure *meso*-**5**. Pure *d,l*-**5** proved to be indistinguishable (^1H NMR, ^{13}C NMR, IR, MS, TLC, mp, $[\alpha]_D$) from an authentic sample of the natural product, a racemate, and pure *meso*-**5** displayed properties identical (^1H NMR, IR, MS, TLC R_f, mp) to those reported at the time of its initial identification.[5b] Synthetic *d,l*-isochrysohermidin (*d,l*-**5**) exhibited only three singlets in the ^1H NMR (400 MHz) in a variety of solvents ($CDCl_3$, CD_3OD, acetone-d_6, DMSO-d_6, pyridine-d_6, CF_3CO_2D) and proved indistinguishable from natural *d,l*-**5** isolated by crystallization and identified in a single-crystal X-ray structure determination[5] (Table IV). After isolation, the two diastereomers proved remarkably stable to interconversion and could be individually subjected to full characterization without evidence of isomerization (*i.e.* mp: *d,l*-**5** 266–268 °C, *meso*-**5** 207–209 °C) (Tables IV and V). Solutions of pure *d,l*- or *meso*-**5** in $CDCl_3$ (60 °C, 70 h), CD_3OD (60 °C, 48 h), and acetone-d_6

Table V. ^{13}C NMR (100 MHz) of Isochrysohermidin (**5**)[a]

assignment	acetone-d_6	CD_3OD	DMSO-d_6	pyridine-d_5
		d,l-**5**		
$CONCH_3$	172.2	172.9	169.7	171.0
CO_2CH_3	169.2	171.0	168.6	170.3
C4	168.9	168.9	167.9	169.2
C3	98.3	97.9	97.2	98.7
C5	88.0	88.7	86.8	88.4
CO_2CH_3	59.5	60.1	58.8	59.3
OCH_3	53.6	54.0	53.3	53.2
NCH_3	24.2	24.5	24.1	24.5
		meso-**5**		
$CONCH_3$		173.0	169.6	
CO_2CH_3		170.9	168.5	
C4		168.9	167.7	
C3		97.9	97.1	
C5		88.6	86.7	
CO_2CH_3		60.0	58.7	
OCH_3		54.0	53.2	
NCH_3		24.5	23.9	

[a] ^{13}C NMR of separated and pure samples of *d,l*- and *meso*-**5**.

proved stable to interconversion while slow (<10%) isomerization was observed in DMSO-d_6 (25 °C, 24 h). Exposure of pure *d,l*- or *meso*-**5** to CF_3CO_2D led to slow interconversion to provide a 1:1 mixture of the two diastereomers (24 h, 25 °C) with detectable ring open intermediates occasionally observable in the ^1H NMR.

Interstrand DNA Cross-Linking. The two orthogonally positioned carbinolamides symmetrically housed within the 3,3′-bis-2-oxo-3-pyrroline skeleton suggested it may represent an ideal candidate for interstrand DNA cross-linking through two sequential reversible carbinolamide exchange reactions with nucleophilic sites within duplex DNA. Moreover, the unusual stability characteristic of such five-membered amido acetal and amido aminal adducts could be anticipated from the behavior of simpler agents[19] and was supported through empirical observation of the exceptionally slow *d,l*- and *meso*-**5** interconversion. Encouraged by these observations, we have examined the potential DNA cross-linking properties of the agents and herein provide the initial characterization of their slow but stable formation of interstrand DNA cross-links.

The interstrand DNA cross-linking of *d,l*- and *meso*-**5** was assessed using two different systems to quantitate double-strand versus single-strand DNA separated under denaturing gel electrophoresis conditions following exposure to the agents. The first simply involves alkaline agarose gel electrophoresis of linear ΦX174 DNA[38] under denaturing conditions (0.7% agarose gel with 0.03 M NaOH) after agent treatment.[39] In the event of DNA cross-linking, fluorescence visualization should reveal two bands. The faster moving, lower molecular weight band constitutes the unmodified or modified but noncross-linked single-stranded DNA, and the slower moving band constitutes the cross-linked duplex DNA (5386 base pairs). Concurrent control studies conducted employing photochemical induced psoralen interstrand DNA cross-linking of the linear ΦX174 DNA ensure the accurate assignment of the two observed bands. The second protocol[40] involves treatment of ^{32}P singly 5′-end labeled w794 DNA[41,42,43] with the agents. High-resolution polyacrylamide gel electrophoresis (PAGE) under denaturing conditions followed by

(38) *Pst*I digest of double-stranded ΦX174 DNA was purchased from New England Biolabs.

(39) Cech, T. R. *Biochemistry* **1981**, *20*, 1431.

(40) Boger, D. L.; Palanki, M. S. S. *J. Am. Chem. Soc.* **1992**, *114*, 9318. Boger, D. L.; Johnson, D. S.; Palanki, M. S. S.; Kitos, P. A.; Chang, J.; Dowell, P. *BioMed. Chem.* **1993**, *1*, 27.

(41) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.

(42) Ambrose, C.; Rajadhyaksha, A.; Lowman, H.; Bina, M. *J. Mol. Biol.* **1989**, *210*, 255.

(43) This was accomplished by EtOH precipitation of the DNA, removal of the EtOH supernatant containing unreacted agent, and redissolution of the agent in aqueous buffer under the original reaction conditions.

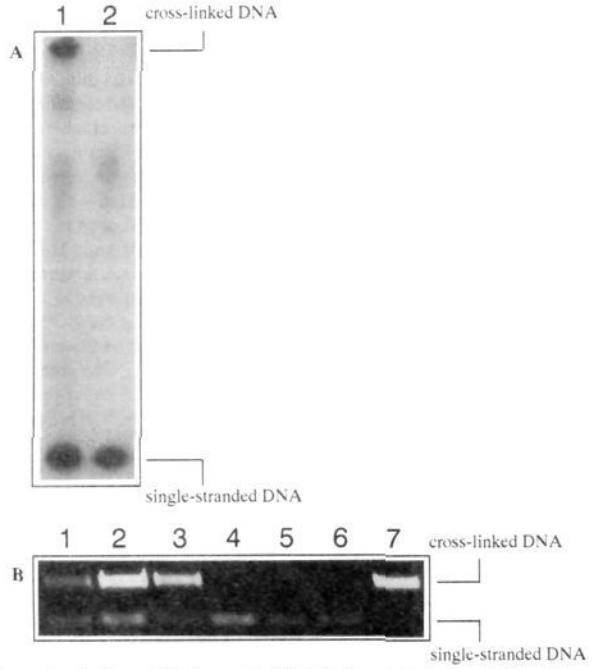


Figure 1. A: Cross-linking study of *d,l*-**5** with w794 duplex DNA. DNA-agent incubation at 25 °C (7 days, pH 7.4) and removal of unbound agent followed by denaturing 8% PAGE and autoradiography. Lane 1, 0.075 *d,l*-**5**; lane 2, untreated DNA. B: Cross-linking study of *d,l*-**5** with duplex linear ΦX174 DNA (*Pst*I digest, 1×10^{-8} M) at 25 °C (4 days, *pH 8.7) and denaturing (90 °C, 2 min) followed by gel electrophoresis.* The gel electrophoresis was conducted at 42 mA (5 h) at 4 °C on a 0.7% agarose gel containing 0.1 μg/μL ethidium bromide and 0.03 M NaOH. Lane 1, 0.075 M *d,l*-**5**, pH 6.0; lane 2, 0.075 M *d,l*-**5**, pH 8.70; lane 3, 0.075 M *d,l*-**5**, pH 4.2; lane 4, untreated DNA, pH 6.0; lane 5, untreated DNA, pH 8.7; lane 6, untreated DNA, pH 4.2; lane 7, psoralen (0.01 M) cross-linking (365-nm *hν*, 1 h, 25 °C).

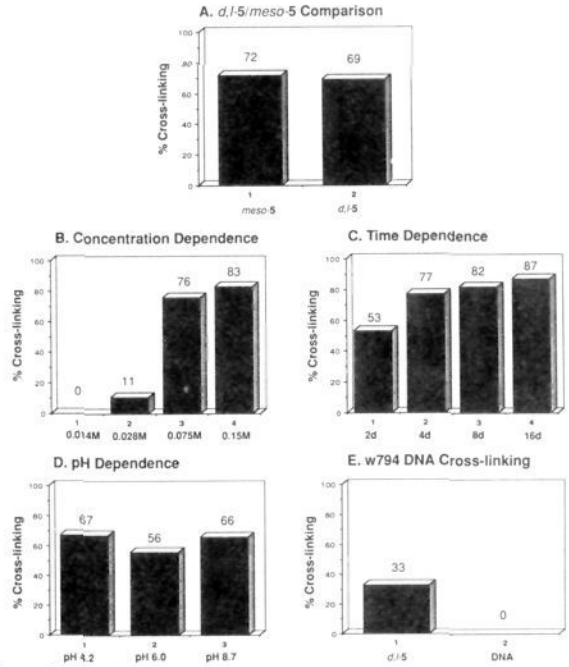


Figure 2. A–D: Cross-linking studies of *d,l*- and *meso*-**5** with duplex linear ΦX174 DNA (*Pst*I digest, 1×10^{-8} M) at 25 °C with denaturing (90 °C, 2 min) followed by denaturing gel electrophoresis. The gel electrophoresis was conducted at 42 mA (5 h) at 4 °C on a 0.7% agarose gel containing 0.1 μg/μL ethidium bromide and 0.03 M NaOH: A, Incubation (*25 °C, 4 days, pH 8.7*) with 0.075 M *d,l*- and *meso*-**5**; B, Incubation (25 °C, 4 days, pH 8.7); C, Incubation (25 °C, 4 days, pH 8.7) with 0.075 M *d,l*-**5**; D, Incubation (25 °C, 4 days) with 0.075 M *d,l*-**5**. E: Cross-linking study of *d,l*-**5** with w794 duplex DNA. Incubation (25 °C, 7 days, pH 7.4) with 0.075 M *d,l*-**5** and removal of unbound agent followed by denaturing 8% PAGE and autoradiography.

autoradiography should provide two readily separable bands in the event of interstrand DNA cross-linking. The faster moving, low molecular weight band constitutes the unmodified or modified but noncross-linked end-labeled single-strand DNA (144 bases), and the much slower moving band constitutes the cross-linked duplex DNA (w794 template-labeled DNA cross-link, 7394 base pairs). Psoralen served as a control for characterization of an authentic cross-linking reaction.[40]

In both protocols, *d,l*- and *meso*-**5** proved to be effective at providing stable interstrand DNA cross-links (Figure 1). In side by side comparisons, no significant distinction was observed in the relative efficiency of cross-linking by *d,l*- and *meso*-**5** (Figure 2A). Both agents exhibited well-defined concentration (Figure 2B) and time-dependent (Figure 2C) cross-linking, and the rate or extent of cross-linking was shown to exhibit a significant pH dependence. Acidic (pH 4.2) and basic (pH 8.7) conditions proved more effective than near-neutral conditions (pH 6–7) (Figure 2D). Presumably, this reflects the unusual stability of the five-membered carbinolamides under the mild, near-neutral pH reaction conditions. It is likely that the more acidic or basic *reaction conditions both catalyze slow, reversible ring-opening* reactions of *d,l*-**5**, and the putative intermediate amido ketones bearing an especially electrophilic carbonyl react with nucleophilic sites within duplex DNA. Although acid-catalyzed acyliminium ion generation may lead to DNA modification, such a mechanism is unlikely to be operative under the basic conditions of pH 8.7 and the extent of cross-linking by **5** would be expected to follow a simple pH dependence (pH $4.2 > 6 \gg 8.7$ versus $4.2 > 6 < 8.7$). Likely candidates for the nucleophilic sites within duplex DNA responsible for the DNA cross-linking include the guanine C2 amine located in the minor groove as well as the adenine C9 and cytosine C4 amines located in the major groove.

A detailed study of the *d,l*- and *meso*-isochrysohermidin interstrand DNA cross-linking reactions and an assessment of their biological properties are in progress, and the results of the ongoing studies will be reported in due course.

Experimental Section

5,5'-Dimethoxy-3,3',6,6'-tetrakis(methoxycarbonyl)-4,4'-bi-1,2-diazine (8). Method A: A solution of **6**[11] (84 mg, 0.48 mmol) in anhydrous $CHCl_3$ (1.0 mL, 0.5 M) containing 4-Å molecular sieves (5 wt equiv, activated) was stirred under Ar (5 min). The solution was treated with 3,6-bis(methoxycarbonyl)-1,2,4,5-tetrazine[8] (**7**, 428 mg, 2.16 mmol, 4.5 equiv) and allowed to stir for 20 min at 22 °C under Ar. After the vigorous evolution of N_2 subsided, the solution was allowed to stir at 60 °C under Ar for 5 days. The reaction mixture was concentrated under reduced pressure. Chromatography (SiO_2, 4 × 12 cm, Et_2O) afforded **8** as a pale-yellow solid (140 mg, 217 mg theoretical, 65%): mp 124–125 °C (Et_2O); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.12 (s, 3H, OMe), 3.89 (s, 3H, CO_2Me), 3.82 (s, 3H, CO_2Me); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 164.4 (e, C3 *C*O_2Me), 163.8 (e, C6 *C*O_2Me), 155.9 (e, C5), 150.4 (e, C4), 147.3 (e, C3), 125.8 (e, C6), 61.6 (o, OMe), 53.7 (o, CO_2*Me*), 53.4 (o, CO_2*Me*); UV ($CHCl_3$) λ_{max} 248 nm (ε 58 000); IR (KBr) ν_{max} 3745, 1735, 1697, 1438, 1385, 1288, 1249, 1211, 1098, 1054 cm^{-1}; EIMS *m/e* (relative intensity) 450 (M^+, 4), 378 (14), 377 (95), 325 (35), 211 (91), 179 (39), 59 (base); CIMS (2-methylpropane) *m/e* 451 (M + H^+, base); EIHRMS *m/e* 450.1021 ($C_{18}H_{18}N_4O_{10}$ requires 450.1023). Anal. Calcd for $C_{18}H_{18}N_4O_{10}$: C, 48.01; H, 4.03; N, 12.44. Found: C, 47.72; H, 3.93; N, 12.22.

Method B: A solution of **6**[11] (400 mg, 2.3 mmol) in anhydrous C_6H_6 (4.6 mL, 0.5 M) was treated with **7**[8] (1.4 g, 6.9 mmol, 3.0 equiv) and allowed to stir at 22 °C under Ar for 20 min. After the vigorous evolution of N_2 subsided, the solution was allowed to stir at 80 °C under Ar for 72 h. The reaction mixture was treated with glacial HOAc (4.6 mL) and allowed to stir at 80 °C under Ar for 60 h. The reaction mixture was cooled and concentrated under reduced pressure. Chromatography (SiO_2, 5 × 14 cm, Et_2O) afforded **8** as a pale-yellow solid (575 mg, 1.03 g theoretical, 56%).

Table VI

	scale (mmol)	time since Zn activation	% 9
1	0.8	24 h	52
2	0.6	3 weeks	40
3	0.5	5 weeks	40
4	0.5	6 weeks	32
5	0.7	9 weeks	10

4,4'-Dimethoxy-2,2',5,5'-tetrakis(methoxycarbonyl)-3,3'-bipyrrole (9). A slurry of glacial HOAc (11.8 mL, 0.085 M), activated[18] Zn dust (1.3 g, 20 mmol, 20 equiv), and **8** (450 mg, 1.0 mmol) was allowed to stir under Ar at 22 °C for 8 h. Additional activated Zn dust (1.3 g, 20 mmol, 20 equiv) was added, and the mixture was stirred under Ar for an additional 14 h at 22 °C. The reaction mixture was diluted with CH_2Cl_2 and filtered through Celite. The crude mixture was made basic (pH 8) with the addition of 10% aqueous NH_4OH. The layers were separated, and the aqueous layer was extracted with EtOAc (5 × 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 3 × 15 cm, Et_2O) afforded **9** as a pale-yellow solid (290 mg, 424 mg theoretical, 68%): mp 199–200 °C (*i*-PrOH, colorless needles); ^1H NMR ($CDCl_3$, 500 MHz) δ 9.44 (bs, 1H, NH), 3.98 (s, 3H, OMe), 3.78 (s, 3H, CO_2Me), 3.72 (s, 3H, CO_2Me); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 160.6 (e, C2 *C*O_2Me), 160.3 (e, C5 *C*O_2Me), 150.7 (e, C4), 120.6 (e, C3), 114.1 (e, C2), 112.6 (e, C5), 62.0 (o, OMe), 51.8 (o, CO_2*Me*), 51.8 (o, CO_2*Me*); UV ($CHCl_3$) λ_{max} 282 nm (ϵ 46 000); IR (KBr) ν_{max} 3315, 3286, 2956, 1727, 1705, 1556, 1491, 1440, 1303, 1267, 1247, 1200, 1143, 1110, 1017, 957, 784 cm^{-1}; EIMS *m/e* 424 (M^+, base); CIMS (2-methylpropane) *m/e* 425 (M + H^+, base); EIHRMS *m/e* 424.1119 ($C_{18}H_{20}N_2O_{10}$ requires 424.1118). Anal. Calcd for $C_{18}H_{20}N_2O_{10}$: C, 50.95; H, 4.75; N, 6.60. Found: C, 50.79; H, 4.88; N, 6.54.

This reductive ring contraction reaction proved sensitive to the quality of Zn employed and was found to proceed best with freshly activated[18] Zn (Table VI).

4,4'-Dimethoxy-2,2',5,5'-tetrakis(methoxycarbonyl)-1,1'-dimethyl-3,3'-bipyrrole (10). A suspension of NaH (110 mg, 2.76 mmol, 2.75 equiv washed free of oil with 3 × 8 mL of pentane) in DMF (4.0 mL, 0.1 M) was cooled to −10 °C, treated dropwise with a solution of **9** (425 mg, 1.0 mmol) in DMF (6.0 mL), and allowed to warm to 22 °C over 1 h. The reaction mixture was recooled to −10 °C, treated dropwise with CH_3I (1.5 g, 10 mmol, 10 equiv), and allowed to warm to 22 °C over 12 h. The mixture was partitioned between EtOAc and H_2O, and the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 4 × 15 cm, 80% Et_2O–hexane) afforded **10** as a white solid (446 mg, 453 mg theoretical, 98%): mp 119–120 °C (*i*-PrOH, colorless prisms); ^1H NMR ($CDCl_3$, 200 MHz) δ 4.16 (s, 3H, NMe), 3.91 (s, 3H, OMe), 3.63 (s, 3H, CO_2Me), 3.60 (s, 3H, CO_2Me); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 161.7 (e, C2 *C*O_2Me), 161.4 (e, C5 *C*O_2Me), 150.6 (e, C4), 124.3 (e, C3), 117.2 (e, C2), 113.7 (e, C5), 62.1 (o, OMe), 51.5 (o, CO_2*Me*), 51.4 (o, CO_2*Me*), 34.9 (o, NMe); UV ($CHCl_3$) λ_{max} 286 nm (ϵ 22 000); IR (KBr) ν_{max} 2955, 1719, 1483, 1458, 1433, 1414, 1399, 1350, 1283, 1241, 1206, 1116, 1032 cm^{-1}; EIMS *m/e* 452 (M^+, base); CIMS (2-methylpropane) *m/e* 453 (M + H^+, base); EIHRMS *m/e* 452.1431 ($C_{20}H_{24}N_2O_{10}$ requires 452.1431). Anal. Calcd for $C_{20}H_{24}N_2O_{10}$: C, 53.10; H, 5.35; N, 6.19. Found: C, 52.89; H, 5.50; N, 6.17.

4,4'-Dimethoxy-1,1'-dimethyl-3,3'-bipyrrole-2,2',5,5'-tetracarboxylic Acid (11). A solution of **10** (206 mg, 0.46 mmol) in 3:1:1 THF–MeOH–H_2O (3.0 mL, 0.15 M) was treated with LiOH (116 mg, 2.8 mmol, 6 equiv) and was allowed to stir under Ar at 22 °C (72 h). The reaction mixture was partitioned between Et_2O and H_2O, and the aqueous layer was made acidic (pH 1) with the addition of 10% aqueous HCl. The aqueous layer was extracted with EtOAc (6 × 30 mL), and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to afford nearly pure **11**. Recrystallization from *i*-PrOH–Et_2O afforded **11** as a white solid (180 mg, 180 mg theoretical, 100%): mp 190–192 °C dec; ^1H NMR (acetone-d_6, 200 MHz) δ 4.14 (s, 3H, NMe), 3.66 (s, 3H, OMe); ^{13}C NMR (CD_3OD, 50 MHz) δ 164.2 (e, C2 CO_2H), 163.6 (e, C5 CO_2H), 152.2 (e, C4), 126.6 (e, C3), 118.5 (e, C2), 115.5 (e, C5), 47.7 (o, OMe), 35.4 (o, NMe); UV (CH_3CN) λ_{max} 288 nm (ϵ 22 700); IR (KBr) ν_{max} 3855, 3745, 3630, 3423, 2966, 2616, 1685, 1526, 1444, 1419, 1352, 1262, 1207, 1114 cm^{-1}; FABHRMS (NBA) *m/e* 397.0810 ($C_{16}H_{16}N_2O_{10}$ + H^+ requires 397.0883). Anal. Calcd for $C_{16}H_{16}N_2O_{10}$: C, 48.49; H, 4.07; N, 7.07. Found: C, 48.09; H, 4.44; N, 7.38.

4,4'-Dimethoxy-5,5'-bis(methoxycarbonyl)-1,1'-dimethyl-3,3'-bipyrrole-2,2'-dicarboxylic Acid (12). From 11: a solution of **11** (40 mg, 0.1 mmol) in trifluoroacetic anhydride (0.50 mL, 0.2 M) was allowed to stir under N_2 at 22 °C for 45 min. The reaction mixture was diluted with pentane (5 mL), decanted from the precipitated residue, and concentrated under reduced pressure to afford crude **24**: ^1H NMR (acetone-d_6, 200 MHz) δ 4.20 (s, 3H, NMe), 3.70 (s, 3H, OMe); IR (KBr) ν_{max} 3444, 1794, 1765, 1677, 1438, 1421, 1149, 1050 cm^{-1}. A solution of crude **24** in THF (3 mL) was transferred to a 7 dram vial, treated with excess CH_2N_2 in Et_2O (25 mL), and allowed to stand at 22 °C open to the air for 12 h. A solution of the crude product in THF (30 mL) and H_2O (30 mL) was stirred at 22 °C for 2 h open to the air before the mixture was treated with saturated aqueous $NaHCO_3$ (10 mL) and allowed to stir for an additional 10 min. The mixture was washed with Et_2O (2 × 30 mL), and the aqueous layer was made acidic (pH 1) with the addition of 1 N aqueous HCl and extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was recrystallized from *i*-PrOH–Et_2O to afford **12** as a white solid (36 mg, 43 mg theoretical, 83%): mp 175–177 °C dec; ^1H NMR (DMSO-d_6, 400 MHz) δ 4.02 (s, 3H, NMe), 3.81 (s, 3H, OMe), 3.52 (s, 3H, CO_2Me); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.8 (e, CO_2H), 149.9 (e, *C*O_2Me), 149.8 (e, C4), 124.9 (e, C3), 116.1 (e, C2), 113.6 (e, C5), 61.7 (o, OMe), 51.5 (o, CO_2*Me*), 34.8 (o, NMe); UV (THF) λ_{max} 280 nm (ϵ 24 800); IR (KBr) ν_{max} 3418, 2923, 1720, 1655, 1443, 1384, 1261, 1101, 800 cm^{-1}; FABHRMS (NBA) *m/e* 424.1118 ($C_{18}H_{20}N_2O_{10}$ requires 424.1118). Anal. Calcd for $C_{18}H_{20}N_2O_{10}$: C, 50.95; H, 4.75; N, 6.60. Found: C, 51.30; H, 5.07; N, 6.50.

From 21: a solution of **21** (10 mg, 0.017 mmol) in 1:1 THF–MeOH (1.0 mL, 0.02 M) was treated with aqueous 5 M HCO_2NH_4 (83 μL, 0.41 mmol, 25 equiv) and 10% Pd-C (2.5 mg, 0.25 wt equiv) and was allowed to stir under N_2 at 22 °C. After 48 h, the reaction mixture was filtered through Celite and partitioned between 10% aqueous $NaHCO_3$ and Et_2O. The aqueous layer was made acidic (pH 1) with the addition of 10% aqueous HCl and was extracted with EtOAc (4 × 15 mL), dried (Na_2SO_4), and concentrated under reduced pressure to afford **12** (7 mg, 7 mg theoretical, 100%) as a white solid identical in all respects with the material prepared from **11**.

Isochrysohermidin (5). A solution of **12** (40.0 mg, 0.094 mmol) in collidine (30 mL), H_2O (90 mL), and 2-propanol (15 mL) containing Rose Bengal (800 μg, 7.9 × 10^{-4} mmol, 8 mequiv) in a Pyrex tube was treated with a steady stream of O_2 (5 psi). The solution was irradiated with a Hanovia high-pressure mercury lamp (450 W) through a uranium yellow glass filter (transmits > 330 nm) at 22 °C for 1 h. The reaction mixture was partitioned between 10% aqueous HCl and EtOAc. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 1 × 10 cm, EtOAc) afforded 35 mg of product (R_f 0.2–0.5, EtOAc). The product which contained a mixture of *d,l*- and *meso*-**5** as well as ring-opened isomers was dissolved in hot EtOAc which was allowed to slowly concentrate (recrystallization of the mother liquor was repeated two times) to afford *d,l*-**5** as a white solid (14 mg, 38 mg theoretical, 37%; typically 37–43%). The mother liquor was concentrated and the residue purified by chromatography (SiO_2, 1 × 10 cm, EtOAc, 3×) to afford pure *meso*-**5** (3 mg, 38 mg theoretical, 8%). For *d,l*-**5**: mp 266–268 °C (EtOAc, colorless needles), lit[5c] mp 265–268 °C (EtOAc); ^1H NMR ($CDCl_3$, 400 MHz) δ 6.62 (bs, 1H, OH), 3.98 (s, 3H, OMe), 3.83 (s, 3H, CO_2Me), 2.84 (s, 3H, NMe); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.7 (e, *C*ONMe), 168.6 (e, *C*O_2Me), 167.9 (e, C4), 97.2 (e, C3), 86.8 (e, C5), 58.8 (o, CO_2*Me*), 53.3 (o, OMe), 24.1 (o, NMe); IR (KBr) ν_{max} 3478, 3408, 3187, 1760, 1740, 1700, 1684, 1644, 1393, 1363, 1253, 1147, 1052 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 533.0178 ($C_{16}H_{20}N_2O_{10}$ + Cs^+ requires 533.0172).

For *meso*-**5**: mp 207–209 °C (EtOAc, white powder), lit[5b] mp 207–209 °C (1:1, EtOAc–Et_2O); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.65 (bs, 1H, OH), 3.98 (s, 3H, OMe), 3.87 (s, 3H, CO_2Me), 2.78 (s, 3H, NMe); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.6 (e, *C*ONMe), 168.5 (e, *C*O_2Me), 167.7 (e, C4), 97.1 (e, C3), 86.7 (e, C5), 58.7 (o, CO_2*Me*), 53.2 (o, OMe), 23.9 (o, NMe); IR (KBr) ν_{max} 3413, 3180, 2954, 1753, 1730, 1706, 1692, 1638, 1440, 1366, 1307, 1253, 1146, 1053, 938 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 533.0172 ($C_{16}H_{20}N_2O_{10}$ + Cs^+ requires 533.0172).

2,2'-Bis((benzyloxy)carbonyl)-4,4'-dimethoxy-5,5'-bis(methoxycarbonyl)-1,1'-dimethyl-3,3'-bipyrrole (21). A solution of **10** (95 mg, 0.21 mmol) in 3:1:1 THF–MeOH–H_2O (1.0 mL, 0.21 M) was treated with LiOH (17.6 mg, 0.42 mmol, 2.0 equiv) and allowed to stir under N_2 at 22 °C for 7 days. The reaction mixture was partitioned between Et_2O

and H_2O, and the aqueous layer was made acidic (pH 1) with the addition of 10% aqueous HCl. The aqueous layer was extracted with EtOAc (6 × 15 mL), and the combined organic layers were dried (Na_2SO_4) and concentrated to afford 110 mg of a crude mixture of acids. A solution of this crude mixture of acids in DMF (2.0 mL, 0.1 M) was treated with K_2CO_3 (160 mg, 1.16 mmol, 5.5 equiv), n-Bu_4NI (0.80 mg, 0.02 mmol, 0.1 equiv), and $PhCH_2Br$ (198 mg, 1.16 mmol, 5.5 equiv) and allowed to stir under N_2 at 22 °C for 48 h. The reaction mixture was partitioned between Et_2O and H_2O. The aqueous layer was extracted with EtOAc (3 × 15 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 2 × 14 cm, 30% Et_2O–hexane) afforded **21** (37 mg, 127 mg theoretical, 29%), **22** (47 mg, 37%), and **23** (40 mg, 31%).

For **21**: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.25 (m, 3H, Ar), 6.96 (m, 2H, Ar), 4.94 (s, 2H, CH_2), 4.00 (s, 3H, NMe), 3.89 (s, 3H, OMe), 3.50 (s, 3H, CO_2Me); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 161.0 (e, CO_2Me), 160.5 (e, CO_2Bn), 150.4 (e, C4), 134.9 (e), 128.3 (o), 128.2 (o), 128.1 (o), 123.6 (e, C3), 117.3 (e, C2), 114.6 (e, C5), 66.4 (e, CH_2), 62.2 (o, OMe), 51.5 (o, CO_2Me), 35.0 (o, NMe); IR (KBr) ν_{max} 2966, 2919, 1712, 1707, 1443, 1396, 1272, 1226, 1108 cm^{-1}; FABHRMS (NBA–CsI) m/e 737.1111 ($C_{32}H_{32}N_2O_{10}$ + Cs^+ requires 737.1111).

For **23**: mp 93–95 °C (i-PrOH, colorless prisms); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.45 (m, 2H, Ar), 7.36 (m, 3H, Ar), 5.36 (s, 2H, CH_2), 4.14 (s, 3H, NMe), 3.62 (s, 3H, OMe), 3.49 (s, 3H, CO_2Me); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.6 (e, CO_2Me), 160.5 (e, CO_2Bn), 150.6 (e, C4), 135.9 (e), 128.5 (o), 128.2 (o), 128.1 (o), 124.3 (e, C3), 117.2 (e, C2), 113.6 (e, C5), 66.2 (e, CH_2), 62.3 (o, OMe), 51.7 (o, CO_2Me), 35.1 (o, NMe); IR (KBr) ν_{max} 2979, 1754, 1750, 1480, 1397, 1227, 1144, 1098, 1044, 971 cm^{-1}; FABHRMS (NBA–CsI) m/e 737.1121 ($C_{32}H_{32}N_2O_{10}$ + Cs^+ requires 737.1111).

The structure of **23** was established unambiguously in a single-crystal X-ray structure determination conducted with colorless prisms grown from i-PrOH.[26]

For **22**: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.45 (m, 2H, Ar), 7.34 (m, 3H, Ar), 7.15 (m, 3H, Ar), 6.97 (m, 2H, Ar), 5.36 (s, 2H, CH_2), 5.01 (s, 2H, CH_2), 4.19 (s, 3H, NMe), 4.00 (s, 2H, NMe), 3.89 (s, 3H, OMe), 3.60 (s, 3H, OMe), 3.51 (s, 3H, CO_2Me), 3.44 (s, 3H, CO_2Me).

4,4'-Dimethoxy-2,2'-bis(methoxycarbonyl)-1,1'-dimethyl-3,3'-bipyrrole-5,5'-dicarboxylic Acid (20). A solution of **23** (14 mg, 0.023 mmol) in 1:1 THF–MeOH (0.5 mL, 0.05 M) was treated with aqueous 5 M HCO_2NH_4 (0.12 mL, 0.58 mmol, 25 equiv) and 10% Pd-C (3.5 mg, 0.25 wt equiv) and was allowed to stir under N_2 at 22 °C. After 48 h, the reaction mixture was filtered through Celite and partitioned between 10% aqueous $NaHCO_3$ and Et_2O. The aqueous layer was made acidic (pH 1) with the addition of 10% aqueous HCl and extracted with EtOAc (4 × 15 mL), dried (Na_2SO_4), and concentrated under reduced pressure to afford **20** (9.6 mg, 9.8 mg theoretical, 98%): ^1H NMR (acetone-d_6, 400 MHz) δ 4.14 (s, 3H, NMe), 3.64 (s, 3H, OMe), 3.62 (s, 3H, CO_2-Me).

DNA Cross-Linking Studies. Protocol A: Eppendorf tubes containing singly ^{32}P 5'-end-labeled double-stranded w794 DNA prepared exactly as previously detailed[41] (18 μL, in TE buffer at pH 7.4) were treated with *d,l-* and *meso*-**5** in a solution of DMSO (2 μL, 0.75 M; 0.075 M final concentration unless otherwise specified). The reaction was mixed by vortexing and brief centrifugation and subsequently incubated (22 °C, 4 days, pH 8.7 unless otherwise specified). The DNA was separated from unbound agent by EtOH precipitation of the DNA. The EtOH precipitation was conducted by adding tRNA as a carrier (1 μL, 10 μg/μL), a buffer solution containing salt (0.1 volume, 3 M NaOAc in TE), and –20 °C EtOH (2.5 volumes). The solutions were mixed and chilled at –70 °C in an EtOH–dry ice bath for 30 min. The DNA was reduced to a pellet by centrifugation at 4 °C for 15 min, washed with –20 °C EtOH (70% in TE buffer containing 0.2 M NaCl), and recentrifuged briefly. The pellets were dried in a Savant Speed Vac concentrator and resuspended in TE buffer (18 μL). The buffer solutions of cross-linked and untreated DNA were treated with formamide dye solution, were warmed at 100 °C for 2 min, and were placed in an ice bath, and the supernatant (2.4 μL) was loaded onto a gel. Gel electrophoresis was conducted on a denaturing 8% PAGE gel (19:1 acrylamide–*N,N'*-methylenebisacrylamide; 8 M urea). The electrophoresis running buffer (TBE) contained Tris base (100 mM), boric acid (100 mM), and $Na_2EDTA{\cdot}2H_2O$ (0.2 mM) dissolved in H_2O. PAGE was prerun for 30 min with formamide dye solution [xylene cyanol FF (0.03%), bromophenol blue (0.03%), and aqueous Na_2EDTA (8.7%, 250 mM)] prior to loading the samples. Autoradiography of dried gels was conducted at –70 °C using Kodak X-Omat AR film and a Picker Spectra intensifying screen. Psoralen (0.01 M) was run as a positive control with cross-linking induced by irradiation at 365 nm for 1 h.

Protocol B: Eppendorf tubes containing the *Pst*I digest of double-stranded ΦX174 DNA (18 μL, TE buffer pH 8.7 unless otherwise specified) were treated with *d,l-* and *meso*-**5** in a solution of DMSO (2 μL, 0.75 M; 0.075 M final concentration unless otherwise specified). The solution was mixed by vortexing and brief centrifugation and subsequently incubated (25 °C, 4 days unless otherwise specified). The buffer solutions of cross-linked and untreated DNA were treated with bromophenol blue dye solution, were warmed at 90 °C for 2 min, and were placed in an ice bath, and the supernatant (9 μL) was loaded onto an alkaline agarose gel. Gel electrophoresis was conducted at 42 mA (5 h) at 4 °C on a 0.7% agarose gel containing 0.03 M NaOH and 0.1 μg/μL ethidium bromide. The electrophoresis running buffer (Keller, 4 °C) contained Tris base (0.4 mM), NaOAc (0.05 mM), $Na_2EDTA{\cdot}2H_2O$ (10 mM), and NaOH (30 mM) dissolved in H_2O. Psoralen (0.01 M, pH 6.0) was run as a positive control with cross-linking induced by irradiation at 365 nm for 1 h. Direct fluorescence quantitation of the DNA in the presence of ethidium bromide was conducted using a Millipore Bio Image 60S RFLP system visualized on a UV (312 nm) transilluminator.

A. Concentration Dependence of Cross-Linking. The concentration-dependence study was conducted as detailed in protocol B with the exception that a range of concentrations of *d,l*-**5** (0.014, 0.028, 0.075, and 0.15 M) were examined.

B. Time Dependence of Cross-Linking. The time-dependence study was conducted as detailed in protocol B with the exception that the length of time for the incubation was varied (2, 4, 8, and 16 days).

C. pH Dependence of Cross-Linking. The pH-dependence study was conducted as detailed in protocol B with the exception that various pH conditions (pH 4.2, 6.0, and 8.7) were examined.

D. Psoralen Cross-Linking. Eppendorf tubes containing *Pst*I digest of double-stranded ΦX174 DNA (18 μL, TE buffer pH 6.0, 1×10^{-8} M) were treated with psoralen in a DMSO solution (2 μL, 0.1 M; 0.01 M final concentration). The reaction was mixed by vortexing and brief centrifugation, was subsequently incubated (25 °C, 30 min), and then was irradiated (365 nm, 1 h). The buffer solutions of cross-linked DNA were treated with bromophenol blue dye solution and warmed at 100 °C for 2 min. The gel electrophoresis was conducted as described in protocol B. Subjection of *d,l*-**5** to the same conditions provided no evidence of DNA cross-linking.

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Supplementary Material Available: Figures showing structure and unit cell packing diagram of **32**, tables of crystal data, collection parameters, bond lengths and angles, atomic coordinates, and isotropic and anisotropic displacement coefficients, and text describing conditions used to collect the data (12 pages). Ordering information is given on any current masthead page.