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Synthesis and Complement Inhibitory Activity of B/C/D-Ring Analogues of the Fungal Metabolite 6,7-Diformyl-3′,4′,4a′,5′,6′,7′,8′,8a′-octahydro-4,6′,7′-trihydroxy-2′,5′,5′,8a′-tetramethylspiro[1′(2′*H*)-naphthalene-2(3*H*)-benzofuran]

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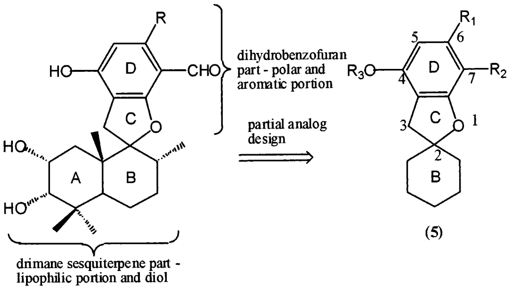
This paper reports the synthesis and the bioassay of 4-methoxy- and 4-hydroxyspiro[benzofuran-2(3*H*)-cyclohexane] partial analogues (**5**) of the complement inhibitory sesquiterpene fungal metabolite 6,7-diformyl-3′,4′,4a′,5′,6′,7′,8′,8a′-octahydro-4,6′,7′-trihydroxy-2′,5′,5′,8a′-tetramethylspiro[1′(2′*H*)-naphthalene-2(3*H*)-benzofuran] (**1a,** K-76) and its silver oxide oxidized product (**1b**, K-76COOH). The described target compounds represent spirobenzofuran B/C/D-ring analogues lacking the A-ring component of the prototype structure. The target compounds were evaluated by the inhibition of total hemolytic complement activity in human serum. It was observed that the structurally simplified analogue 4-methoxyspiro[benzofuran-2(3*H*)-cyclohexane]-6-carboxylic acid (**5a**) exhibited an $IC_{50} = 0.53$ mM similar to the $IC_{50} = 0.57$ mM that was observed for the natural product derivative **1b**. Exhibiting an $IC_{50} = 0.16$ mM, the three-ringed partial structure 6-carboxy-7-formyl-4-methoxyspiro[benzofuran-2(3*H*)-cyclohexane] (**5k**)was found to be the most potent target compound. Like the natural product, **5k** appears to inhibit primarily at the C5 activation step and inhibits both the classical and alternative human complement pathways. Several other analogues inhibited complement activation in vitro at concentrations similar to those required for inhibition by the natural product **1b**.

Introduction

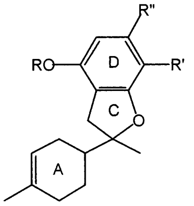
Interest in the development of modulators of the complement cascade has recently increased because of a growing understanding of the role of complement in various disease processes.[1−5] This has led to attempts to identify structurally diverse complement inhibitory natural products and synthetic agents.[7] A microbial metabolite of the fungal species *Stachybotrys complementi* nov. sp. K-76, 6,7-diformyl-3′,4′,4a′,5′,6′,7′,8′,8a′-octahydro-4,6′,7′-trihydroxy-2′,5′,5′,8a′-tetramethylspiro[1′(2′*H*)-naphthalene-2(3*H*)-benzofuran] (**1a**), and its oxidized derivative **1b** have been shown to inhibit complement[6,7] and were examined in a wide variety of in vivo studies.[8−15] Their structure determination[15] and three total syntheses have been reported.[17−19]



(5)

1a, K-76, R=CHO

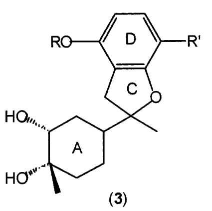
1b, K-76COOH, R=COOH



(2)

R = H, Me, Ph

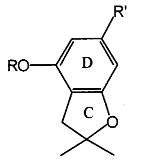
R′ = R″ = H, CHO, COOH



(3)

R = H, Me

R′ = CHO, COOH



(4)

R = H, Me, Ph

R′ = CHO, COOH, tetrazolyl

The reported experimental data demonstrating the complement inhibitory activity of **1b**, its use in a number of animal disease models, and its unique chemical structure make it an interesting drug prototype for further exploration.[7−9,12,14−20] In an attempt to elucidate the essential pharmacophore of compounds **1a** and **1b**, the natural product was used as a topographical model for the design of partial analogues retaining the desired complement inhibitory activity. Structurally, **1a**

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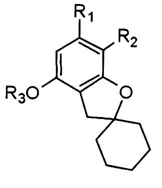
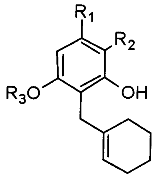
Table 1. Analogues of **1a**



5a-k

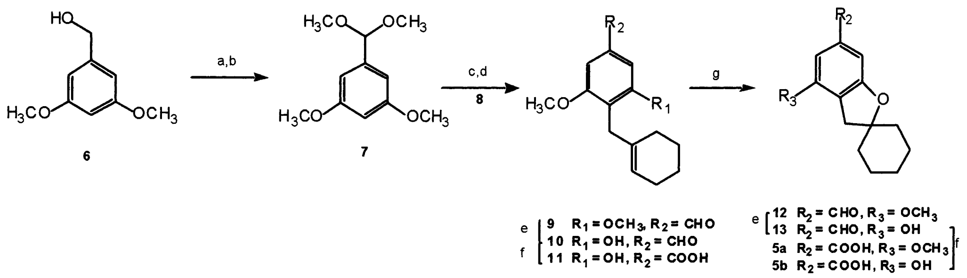


11 and 19

compd	R_1	R_2	R_3	mp (°C)	total hemolysis IC_{50} ± SD (mM)	alternative hemolysis IC_{50} ± SD (mM)
1b	COOH	CHO	H		0.57 ± 0.17, *n* = 9	0.33 ± 0.25, *n* = 2
5a	COOH	H	Me	203−205	0.53 ± 0.19, *n* = 23	2.2 ± 1.2, *n* = 8
5b	COOH	H	H	223−225	1.5 ± 0.21, *n* = 2	3.8 ± 1.3, *n* = 2
5c	R_1···CH_2−O−CO···R_2		Me	132−134		
5d	CHO	COOH	Me	128−130	1.7 ± 0.15, *n* = 3	1.9, *n* = 1
5e	COOH	COOH	Me	188−190	0.80 ± 0.36, *n* = 3	1.2 ± 1.3, *n* = 3
5f	COOH	CHO	Me	132−134	0.16 ± 0.08, *n* = 7	0.73 ± 0.20, *n* = 3
5g[a]	H	COOH	Me	192−194	1.3 ± 0.49, *n* = 10	2.2 ± 1.6, *n* = 3
5h[a]	H	COOH	H	173−174	3.0, *n* = 1	
5i[a]	H	$CONHCH_3$	Me	147−148		
5j	H	CO-1-(4-Me-Pip)	Me	255−256		
5k	H	CONHOH	Me	165−167		
11	COOH	H	Me	152−153	0.82 ± 0.16, *n* = 2	3.5 ± 1.6, *n* = 3
19[a]	H	COOH	Me	161−163	2.1 ± 0.71, *n* = 2	

[a] Synthesis was published earlier.[20a]

Scheme 1[a]



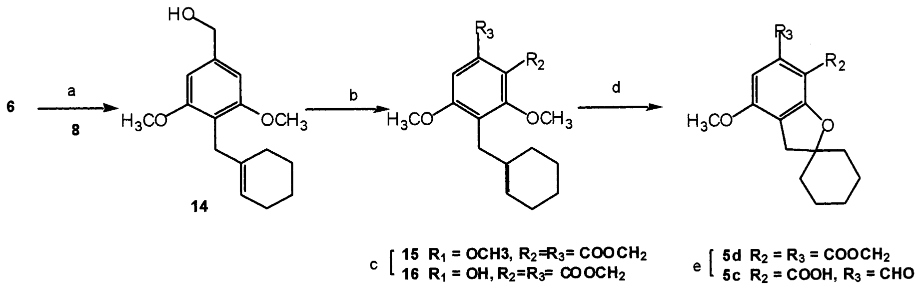
[a] Reagents and conditions: (a) PCC/CH_2Cl_2; (b) *p*-TsOH/MeOH; (c) *n*-BuLi/TMEDA/THF, CuI, 1-bromomethylcyclohexene (**8**); (d) H^+; (e) *t*-BuSLi/HMPA; (f) Ag_2O, aqueous NaOH; (g) Amberlyst 15.

contains a drimane sesquiterpene moiety attached to a 3,5-dihydroxyphthalic dicarboxaldehyde via the 3-phenolic oxygen and the 4-position with the formation of spirobenzofuran ring system. In the natural product model, the A/B-rings constitute the sesquiterpene moiety while the C/D-rings form the benzofuran portion of the molecule. This description defines **1a** as a combination of two chemically distinct regions: a highly functionalized polar aromatic moiety and a relatively lipophilic, alicyclic portion containing a *cis*-diol. A number of ring-limited analogues of K-76 of general structures represented by **2** (A/C/D-ring analogues), **3** (A/C/D-ring analogues), and **4** (C/D-ring analogues) have been synthesized and tested for anticomplement activity.[21] In our preliminary design of the target B/C/D-ring **1a** partial analogues (**5**), the aromatic portion of the model natural product was thought to be essential. Therefore, in a general sense, we planned to introduce into the 3-position of 2,4-dihydroxybenzoic acid the simple six-carbon cycloalkane that would be analogous to the sesquiterpene B-ring of **1a** and further evaluate the necessity for the intact spirofuran C-ring system. The target B/C/D-ring substructure of the natural product model **1a** was envisioned to be synthetically accomplished via acidic cyclization of a partial phenolic B/D-ring intermediate (such as **9**). Functional groups on the aromatic ring proposed in the target analogues were selected to mimic the prototype natural product derivative monocarboxaldehyde/monocarboxylic acid **1b** and its opposite regioisomer. The primary objective of the investigation reported herein was to further define a structural pattern for **1b**-like complement inhibitory activity utilizing simplified and synthetically tractable B/C/D-ring target compounds with the assumption that these could serve as useful tools in gaining a better understanding of the role of complement in disease and injury.

Chemistry

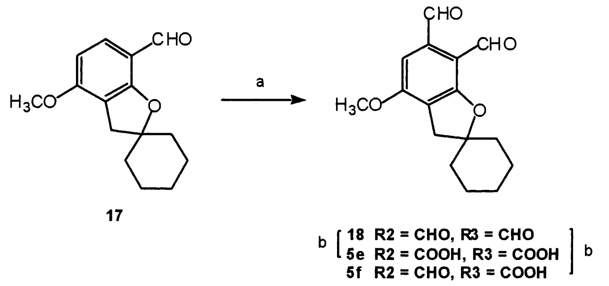
Synthetic routes leading to the target compounds listed in Table 1 are illustrated in Schemes 1−4. Generally, the synthesis of the B/C/D-ring skeleton of **1a** partial analogues was similar to that described before.[21,22a] The crucial synthetic steps envisioned were the coupling of a protected D-ring lithiated resorcinol or resorcylic derivative with the requisite B-ring allylic bromide. Subsequent cyclization of the C-ring would afford the target B/C/D-ring spirodihydrobenzofuran analogues (**5**). The appropriate substitution pattern on the aromatic D-ring was achieved by utilizing either a protected 5-substituted 1,3-dimethoxybenzene derivative followed by addition of an electrophile (Schemes 1 and 2) or by the 7-substituent assisted metalation followed by the addition of an electrophile (Scheme 3)

Scheme 2[a]



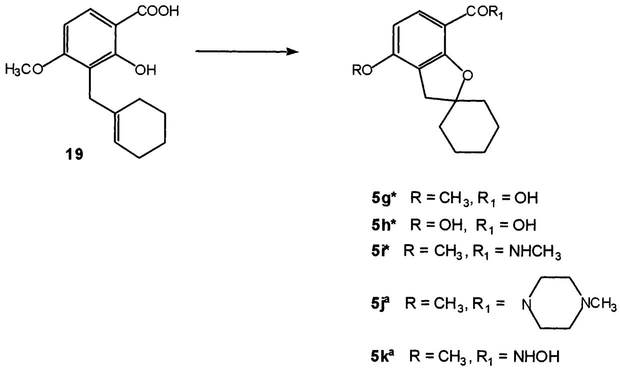
[a] Reagents and conditions: (a) *n*-BuLi/TMEDA/THF, CuI, 1-bromomethylcyclohexene (**8**); (b) *n*-BuLi/TMEDA/hexanes, CO_2; (c) *t*-BuSLi/HMPA; (d) Amberlyst 15; (e) KMnO4/(n-Bu)$_4$NF/THF/aqueous NaOH.

Scheme 3[a]



[a] Reagents and conditions: (a) *n*-BuLi/*N*,*N*,*N'*-trimethylethylenediamine/THF, DMF; (b) AgOH/Ag_2O/EtOH/aqueous KOH, 2 h. The synthesis of compound **16** was previously described.[21a]

Scheme 4[a]



[a] (*) The syntheses of compounds **19** and **5g**,**h**,**i** were previously described.[21a] From **5e**: $SOCl_2$/60 °C/2 h and an excess of amine-R_1/1 h.

or by the derivatization of an appropriately substituted B/D-ring intermediate (Scheme 4).

Methyl 3,5-dimethoxybenzoate was reduced with lithium aluminum hydride to the corresponding benzyl alcohol **6**. Alcohol **6** (Scheme 1) was subsequently oxidized to the corresponding benzaldehyde and protected as its dimethyl acetal **7.** ^{1}H NMR data confirm that the lithiated intermediate of acetal **7** was formed regioselectively at the 4-position. Two magnetically equivalent aromatic protons were observed at the positions 5 and 7 of the aromatic ring system in benzaldehyde **9** and the corresponding dimethyl acetal before hydrolysis following copper(I)-assisted coupling of **7** with 1- bromomethylcyclohexene **8**.[22] Selective phenolic deprotection of **9** to intermediate **10** resulted from treatment with lithium *tert*-butyl thiolate in HMPA. Oxidation with silver oxide in an aqueous solution of sodium hydroxide to carboxylic acid **11** was followed by Amberlyst 15 catalyzed cyclization to target spirodihydrobenzofuran **5a**. Similarly, Amberlyst 15 catalyzed cyclization of **10** led directly to benzaldehyde **12**. Benzaldehyde **12** was demethylated as described above for **9** and oxidized with silver oxide to the target 4-hydroxy-substituted carboxylic acid **5b**.

The 6,7-disubstituted analogues in positions 6 and 7 were synthesized in two ways. Direct copper-assisted coupling of the lithium intermediate of **6** with **8** (Scheme 2) was proven to be successful for insertion of the 1-cyclohexenylmethyl moiety in position 4 of **6** with a slight amount of isomer in position 2 (additional peak in GC). Careful chromatographic purification afforded benzyl alcohol **14**, which after piridinium chlorochromate (PCC) oxidation provided benzaldehyde **9** as an additional proof of major regioorientation of coupling with **8**. Hydroxymethyl-group-directed ortho lithiation of **14** and carboxylation with carbon dioxide afforded lactone **15**, which was regioselectively monodemethylated to phenol **16**. ^{1}H NMR analysis confirmed the regioselectivity of monodemethylation based on the absence of the methoxy group protons at the 7-position (δ 4.03 ppm) present in **15** at lower field than the 4-methoxy substituent (δ 3.88 ppm). Methoxyphenol **16** was cyclized to afford **5c** and selectively monoxidized with potassium permanganate and basic conditions to provide compound **5d**. The second approach to the synthesis of the disubstituted analogues in positions 6 and 7 was via intermediate **17**, which was synthesized as we previously described[22a] and subjected to ortho lithiation (Scheme 3) in the presence of *N*,*N*,*N'*-trimethylethylenediamine followed by DMF formylation yielding 6,7-dialdehyde **18**. The ortho substitution was confirmed using ^{1}H NMR by observing the presence of *o*-dialdehyde protons at δ 10.35 and δ 10.70 ppm. The other isomer, 5,7-diformyl-4-methoxyspiro[benzofuran-2(3*H*)-cyclohexane], was synthesized by an alternative route in our laboratory and exhibited chemical shifts δ 10.13 and δ 10.20 ppm for the *m*-dialdehyde. The dialdehyde **18**, oxidized in ethanolic solution with the combination mixture of silver(I) hydroxide and silver(I) oxide precipitated in situ from silver(I) nitrate solution by the addition of aqueous solution of potassium hydroxide, afforded dicarboxylic acid **5e**. Oxidation of **18** under the same conditions as above, but within limited time of 2 h and in THF solution, provided a low yield (7−14%) of the monocarboxylic acid analogue of **1b** (K-76COOH), compound **5f**.

Partial analogues of compound **1a** substituted in position 7 (compounds **5g**, **5h**, and **5i**) were synthesized from **17** as we previously described,[21a] and their derivatives **5j** and **5k** were prepared from **5c** via acid chloride condensation with the corresponding amines (Scheme 4).

Biological Studies

C5a and C3a Production by Serum Complement. The capacity of the compounds to inhibit the proteolytic release of C5a and C3a in an activated human serum sample was assessed as previously described.[21a] Human serum (73% of the final volume) was equilibrated with varying concentrations of the compounds dissolved in 0.10 M HEPES, 0.15 M NaCl, pH 7.4. Complement activation was initiated by the addition of heat-aggregated IgG, and the samples were incubated for a fixed reaction time of 15 min (previously determined to yield ~90% maximal C5a and C3a production). The C5a[desArg] and C3a[desArg] levels were measured using commercially available kits (Amersham, Chicago, IL). C5a[desArg] and C3a[desArg] lack the carboxy-terminal arginine residues of C5a and C3a, respectively, which are rapidly removed by serum proteases. The fractional inhibition was determined relative to the uninhibited sample (no added compound) and the background serum level of anaphylatoxin (no aggregated IgG).

The natural product **1b** inhibits the production of C5a by activated human serum with an IC_{50} of approximately 3 mM (mean of 4.8 ± 2.5 (SD) mM, $n = 3$) but does not inhibit C3a production at concentrations up to 11 mM as was reported previously.[21a] This confirms that complement inhibition by **1b** occurs predominantly at the C5 activation step as reported by others[7] because inhibition is more effective for C5a than for C3a production and because C3 activation immediately precedes C5 activation in both complement pathways. In early studies, a number of commercially available simple analogues of the aromatic D-ring of **1b** were examined for complement inhibitory activity. One of these, β-resorcylic acid (2,4-dihydroxybenzoic acid), demonstrated weak inhibition of C5a production with an IC_{50} of 22 mM. Similar inhibition of C5a production ($IC_{50} = 21$ mM) was observed using the related compound 2-hydroxybenzoic acid (data not shown). Other structurally related simple analogues (such as 4-hydroxybenzoic acid, 2,4-dimethoxybenzoic acid, 3,5-dihydroxybenzoic acid, 2-hydroxycinnamic acid, and 2-formylbenzoic acid) yielded no significant inhibition of C5a or C3a production over the concentration range tested, typically up to 20 mM (data not shown). These results suggested that the intact dihydrobenzofuran structure may not be strictly required for anticomplement activity, which was confirmed by testing the open-ring intermediate **17**, which inhibited both C5a ($IC_{50} = 8$ mM) and C3a ($IC_{50} = 13$ mM) production. The corresponding closed-ring benzofuran **5g** was somewhat more selective for inhibition at the C5 activation step with IC_{50} values of 5 and 22 mM for the inhibition of C5a and C3a production, respectively (Figure 1). The anticomplement activity of **5g** was thus quite comparable to that of the natural product **1b** in this assay. With the carboxyl group in the 6-position as is found in the partially oxidized natural product **1b**, the analogue **5a** is slightly more effective an inhibitor of C5a production ($IC_{50} = 2.5 \pm 0.25$ (SD) mM, $n = 4$) than **5c** and clearly comparable to **1b**. Finally, the 6-carboxy-7-formyl substitution pattern found in **1b** yields a simplified analogue **5f** that is a better inhibitor of C5a production ($IC_{50} = 0.50$ mM) than the lead compound **1b**.

Complement-Mediated Hemolysis. The capacity of the compounds to inhibit complement-mediated erythrocyte lysis (hemolysis) was assessed as described.[21,23] Antibody-sensitized sheep erythrocytes (Diamedix Corp., Miami, FL), the compounds to be tested, and diluted human serum were incubated 60 min at 37 °C. Cells were separated by centrifugation, and the absorbance at 410 nm of the supernatants was measured to quantify released hemoglobin. Samples were paired with identical controls lacking human serum (complement-independent lysis). Values for complement-independent lysis were subtracted from sample values, and the fractional inhibition was determined relative to the uninhibited (no added compound) sample. None of the compounds reported here yielded significant complement-independent lysis. Results are expressed as the concentration of compound that yields 50% uninhibited lysis (IC_{50}).

As seen in Table 1, the natural product **1b** inhibited total hemolysis with an IC_{50} of 0.57 mM as reported previously by us[21a] and by others[8] using a similar assay. Analogues with a carboxyl group as R_1 and a methyl group as R_3 (specifically compounds **5a**, **5e**, **5f**, and **17**) are comparable to the natural product **1b** in the inhibition of total hemolytic complement (IC_{50} values range from 0.16 to 0.82 mM). Analogue **5b**, which has hydrogen instead of methyl as R_3, has a measurable but reduced capacity to inhibit total hemolysis ($IC_{50} = 1.45$ mM) even though R_3 is hydrogen in the natural product **1b**. A similar effect on anticomplement activity was observed with the substitution of methoxyl for hydroxyl groups in a series of A/C/D-ring analogues of **1b**.[21a] The most potent analogue **5f** retains the substitution pattern of the natural product at R_1 and R_2 but has a methyl group as R_3.

The capacity of the compounds to inhibit the lysis of rabbit erythrocytes by the alternative complement pathway in a buffer containing EGTA and Mg^{2+} was assessed as previously described.[24] Rabbit erythrocytes (Lampire, Pipersville, PA), the compounds to be tested, and human serum diluted in buffer (0.10 M HEPES, 0.15 M NaCl, 5.0 mM $MgCl_2$, 8.0 mM EGTA, pH 7.4) to yield 60% total lysis were incubated for 60 min at 37 °C. The cells were separated by centrifugation, and the supernatants were analyzed as described above. Higher concentrations of human serum are required to achieve adequate lysis of the rabbit erythrocytes by the alternative pathway than are required to lyse antibody-sensitized sheep erythrocytes in the total hemolytic assay. Therefore, it is not appropriate to compare IC_{50} values obtained using the total hemolytic assay with those from the alternative pathway hemolytic assay as a measure of the relative effectiveness of a single compound against the two pathways. Of course, comparisons among the various compounds using either assay are appropriate.

As seen in Table 1, the natural product **1b** inhibited alternative pathway hemolysis with an IC_{50} of 0.33 mM, which is comparable to values previously reported using similar assays.[8,15b] The analogue **5f** with the lowest IC_{50}

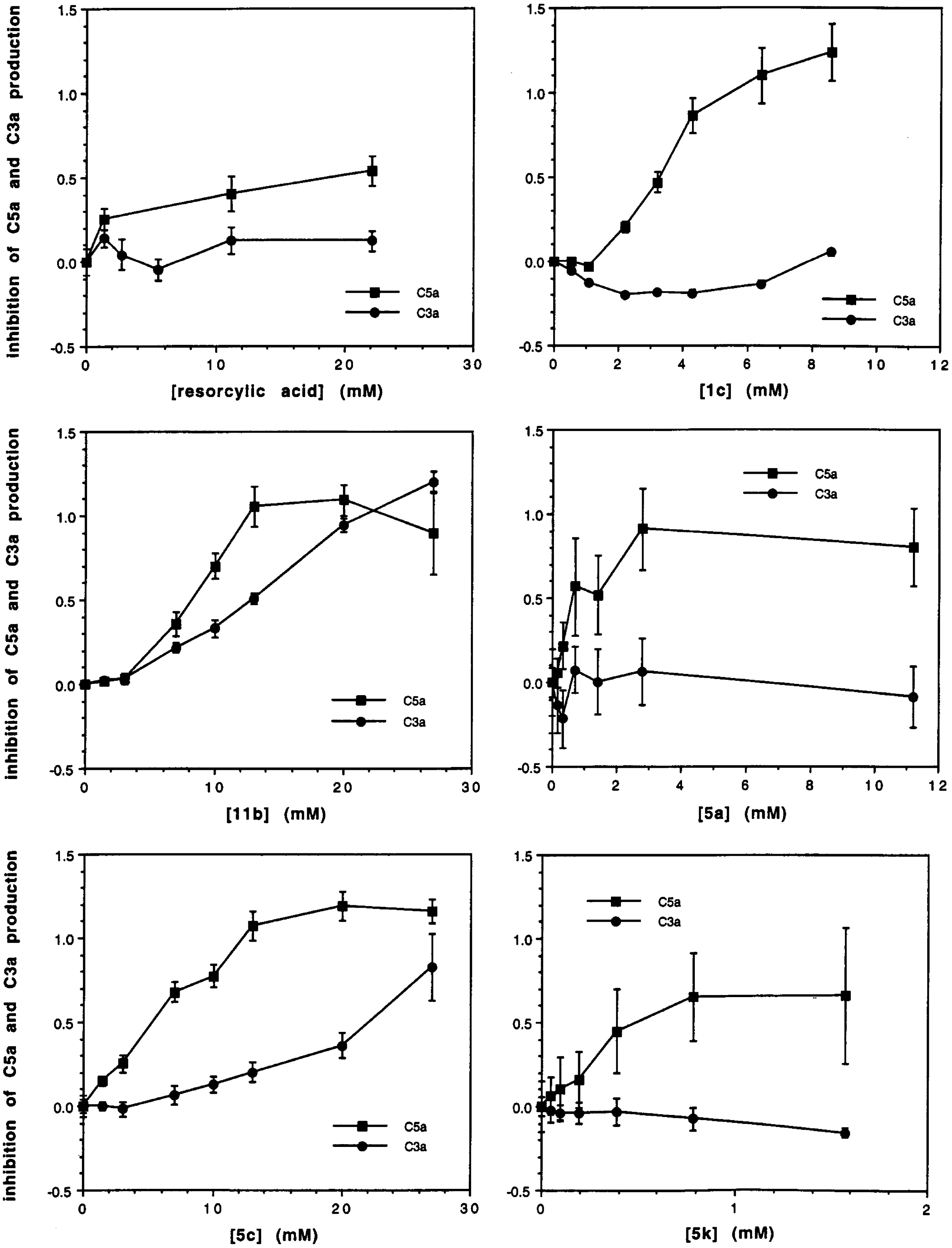


Figure 1. Inhibition of the generation of C5a (squares) and C3a (circles) in human serum activated by heat-aggregated IgG as a function of compound concentration. Error bars represent standard errors ($n = 3$ except for **5f** where $n = 2$) propagated in the normal manner.

in the total hemolytic assay also inhibited alternative hemolysis with an IC_{50} of 0.73 mM, similar to **1b**. The other analogues that were tested required somewhat higher concentrations to inhibit alternative hemolysis with IC_{50} values ranging from 1.2 to 3.8 mM.

Conclusion

A series of B/C/D-ring analogues of the fungal metabolite and known complement inhibitor K-76 (**1a**) and its oxidized analogue **1b** have been synthesized and characterized. The target compounds are greatly simplified analogues of the sesquiterpene natural product, and several exhibit comparable complement inhibitory activity. The analogues, like the natural product, appear to inhibit at the C5 activation step because they inhibit the production of C5a but not C3a in activated human serum. A comparison of the anticomplement activity of analogues with various combinations of carboxyl and formyl groups at the benzofuran 6- and 7-positions suggested the importance of the 6-carboxyl group. The most potent target compound **5f** retains the 6-carboxyl-7-formyl substitution pattern present in **1b**, the partially oxidized monocarboxylic acid form of the natural product. As observed in studies of A/C/D-ring analogues of K-76,[21a] 4-methoxy analogues were somewhat more potent than the corresponding 4-hydroxy derivatives even though a hydroxyl group is found at this equivalent position in the natural product lead. This suggests the potential for non-native substitutions at the 4-position to further improve the potency of future analogues.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 281B spectrophotometer as KBr pellets. The ^{1}H NMR spectra were measured at 299.943 MHz on a Varian VXR 300 spectrometer and, unless stated otherwise, recorded in $CDCl_3$. Chemical shifts are reported in δ (parts per million) units relative to the internal reference tetramethylsilane (TMS). The ^{13}C NMR data were obtained on the Varian VXR 300 spectrometer at 75.429 MHz and are also reported relative to TMS. Electron impact mass spectra (EIMS) data were obtained on a Finnigan 3221-F200 mass spectrometer or a Hewlett-Packard 5985a GC/MS and, for three compounds, on a thermospray LC/MS Vestec model 201 mass spectrometer operated in the positive ion mode using an LC column bypass inlet. The high-resolution mass spectra (HRMS) were obtained on a Finnigan MAT 8200 mass spectrometer (Spectrometry Lab, Department of Chemistry, Massachusetts Institute of Technology). Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA) and are within 0.4% of theory. Dry THF was freshly distilled from benzophenone−sodium still. Other solvents or liquid chemicals described below as dry were freshly distilled or dried prior to use according to the known procedures. Methyl 3,5-dimethoxybenzoate, β-resorcylic acid (2,4-dihydroxybenzoic acid), and β-resorcyclic aldehyde (2,4-dihydroxybenzaldehyde) were commercially available from Aldrich. A sample of K-76COOH was a gift from Otsuka Pharmaceutical, Japan. The column and flash chromatographies were performed, unless stated otherwise, on Kieselgel 60 (0.040−0.063), under gravitational/low pressure, using gradient solvent systems hexanes/ethyl ether/ethyl acetate, or on a Chromatotron. Acetic acid (1%) was added during the chromatographies of carboxylic acids.

3,5-Dimethoxybenzyl Alcohol (6). A solution of methyl 3,5-dimethoxybenzoate (25 g, 128 mmol) in THF (100 mL) was slowly added to a stirring suspension of $LiAlH_4$ (3.6 g, 95 mmol) in THF (400 mL) at 0 °C under a nitrogen atmosphere. After 30 min, the reaction was quenched by the dropwise addition of a saturated solution of Na_2SO_4 until all bubbling ceased. The suspension was filtered, and the filtrate was dried ($MgSO_4$) and concentrated to give 20.5 g (95%) of **6** as white needles: mp 51−52 °C (hexanes) [lit[24] mp 47−48 °C]; ^{1}H NMR δ 2.09 (br s, exc, 1 H), 3.77 (s, 6 H), 4.93 (s, 2 H), 6.37 (t, J = 2 Hz, 1 H), 6.61 (d, J = 2 Hz, 2 H).

3,5-Dimethoxybenzaldehyde Dimethyl Acetal (7). (a) Oxidation. A solution of **6** (6.3 g, 37.5 mmol) in CH_2Cl_2 (50 mL) was added to a stirring suspension of PCC (17.8 g, 83 mmol) and NaOAc (1.4 g, 17 mmol) in CH_2Cl_2 (200 mL). After 16 h, the suspension was quenched with ethyl ether, filtered and concentrated. The crude product was purified by chromatography (Florisil/$CHCl_3$) to afford 5.4 g (87%) of 3,5-dimethoxybenzaldehyde as white needles: mp 46−47 °C [lit[24] mp 45−46 °C]; ^{1}H NMR δ 3.84 (s, 6 H), 6.72 (t, J = 2 Hz, 1 H), 7.03 (d, J = 2 Hz, 2 H), 9.92 (s, 1 H).

(b) Acetal Protection. The aldehyde (3.0 g, 18 mmol) was dissolved in a 2% solution of p-TsOH in MeOH (50 mL), anhydrous $CaCl_2$ (50 mg, 0.45 mmol) was added, and the mixture was stirred for 3 h. The solvent was removed, the residue was dissolved in ethyl ether (50 mL) and washed with a saturated solution of $NaHCO_3$ (3 × 50 mL), and the ether was removed in vacuo. A solution of 5% NaOH (50 mL) and 0.5% $KMnO_4$ (50 mL) was added to the remaining oil, and the mixture was stirred overnight. This mixture was extracted into ether (3 × 50 mL), filtered through a plug of anhydrous K_2CO_3, and concentrated to give 3.2 g (84%) of dimethyl acetal **7** as a light-orange oil: ^{1}H NMR δ 3.37 (s, 6 H), 3.81 (s, 6 H), 5.32 (s, 1 H), 6.44 (t, J = 2 Hz, 1 H), 6.66 (d, J = 2 Hz, 2 H).

4-(1′-Cyclohexenyl)methyl-3,5-dimethoxybenzaldehyde (9). (a) Coupling with 8.[21] To a solution of dimethyl acetal **7** (1.0 g, 4.7 mmol) and TMEDA (1.0 mL, 6.6 mmol) in dry THF (30 mL) at 0 °C, under a nitrogen stream, 1.2 M n-BuLi in hexanes (5.7 mL, 6.8 mmol) was added. The reaction mixture was allowed to warm to room temperature, stirred for 3 h, and then cooled to −70 °C. CuI (1.3 g, 6.8 mmol) was added in one portion. The suspension was warmed to −45 °C, stirred at that temperature for over 1.5 h, and recooled to −70 °C. A solution of **8** (1.2 g, 6.9 mmol) in THF (5 mL) was added dropwise. The reaction mixture was stirred for 24 h at room temperature and quenched by an equal volume of an ice-cooled saturated solution of $NaHCO_3$. The organic phase was extracted with ether (2 × 30 mL), and combined ether extracts were washed exhaustively with a saturated solution of $NaHCO_3$ until the aqueous layer was no longer blue. The organic extracts were filtrated through the plug of K_2CO_3 and concentrated to afford 1.4 g of the crude dimethyl acetal of **9** as an orange oil: ^{1}H NMR δ 1.56 (br m, 4 H), 1.90 (br m, 4 H), 3.24 (br s, 2 H), 3.36 (s, 6 H), 3.81 (s, 6 H), 5.19 (br s, 1 H), 5.33 (s, 1 H), 6.66 (s, 2 H); EIMS m/z 306 (M^+), 275 (100%), 225, 181, 151.

(b) Acetal Deprotection. The crude dimethyl acetal of **9** (1.4 g) was dissolved in a mixture of ethyl ether (50 mL) and 5% aqueous HCl (50 mL) and stirred vigorously for 16 h. The mixture was then saturated with NaCl, and the ether layer was separated, washed with saturated $NaHCO_3$ (2 × 50 mL), filtered through a plug of anhydrous K_2CO_3, and concentrated. The crude product was purified by column chromatography to afford 870 mg (total 71%) of **9** as white needles: mp 75−76 °C (hexanes); IR (cm^{-1}) 2930, 1690, 1590, 1460, 1420, 1380, 1310, 1210, 1140, 1115, 835; ^{1}H NMR δ 1.56 (br m, 4 H), 1.92 (br m, 4 H), 3.31 (br s, 2 H), 3.88 (s, 6 H), 5.20 (br s, 1 H), 7.07 (s, 2 H), 9.89 (s, 1 H); ^{13}C NMR δ 22.4, 23.1, 25.3, 28.9, 31.1, 56.0, 105.0, 120.8, 125.0, 135.5, 135.6, 158.9, 191.9. Anal. ($C_{16}H_{20}O_3$) C, H.

4-(1′-Cyclohexenyl)methyl-3-hydroxy-5-methoxybenzaldehyde (10). A solution of 2.4 M n-BuLi in hexanes (1.2 mL, 2.9 mmol) was added to a stirring solution of t-BuSH (0.35 mL, 3.1 mmol) in dry HMPA (2.5 mL) at 0 °C under a nitrogen atmosphere and stirred for 30 min. This solution was then transferred to a stirring solution of **9** (102 mg, 0.4 mmol) in HMPA (3 mL) at 0 °C. The mixture was stirred at room temperature for 48 h, after which ethyl ether (15 mL) was added and the reaction mixture was extracted with 5% aqueous NaOH (2 × 15 mL). Combined alkali extracts were washed with ether (2 × 15 mL), cooled to 0 °C, acidified with concentrated HCl, and extracted with ether (3 × 15 mL). Drying ($MgSO_4$), concentration, and column chromatography purification afforded 90 mg (93%) of **10** as fine needles: mp 155−156 °C (ether/hexanes); IR (cm^{-1}) 3240, 2930, 1670, 1595, 1515, 1400, 1320, 1210, 1145, 1100, 840, 710; ^{1}H NMR δ 1.60 (br m, 4 H), 1.97 (br m, 4 H), 3.44 (br s, 2 H), 3.87 (s, 3 H), 5.62 (br s, 1 H), 5.83 (br s, exc, 1 H), 7.01−7.04 (m, 2 H), 9.88 (s, 1 H); ^{13}C NMR δ 23.2, 23.8, 25.8, 29.4, 31.6, 56.1, 104.0, 110.4, 120.9, 121.0, 130.2, 136.5, 156.9, 159.6, 168.0. Anal. ($C_{15}H_{18}O_3$) C, H.

4-(1′-Cyclohexenyl)methyl-3-hydroxy-5-methoxybenzoic Acid (11). Compound **10** (246 mg, 1 mmol) was added to a stirred suspension of Ag_2O (350 mg, 1.5 mmol) in 5% aqueous NaOH (8 mL, 1.0 mmol). After being stirred for 16 h, the suspension was filtered and the solid was washed with H_2O. The filtrate was cooled to 0 °C, acidified with concentrated HCl, and extracted with ethyl ether (3 × 25 mL). Drying ($MgSO_4$) and concentration afforded 245 mg (94%) of **11a** as a solid: mp 152−153 °C (ether/acetone); IR (cm^{-1}) 3390, 2930, 1690, 1585, 1425, 1315, 1100, 775; ^1H NMR (acetone-d_6) δ 1.57 (br m, 4 H), 1.96 (br m, 4 H), 3.31 (br s, 2 H), 3.72 (br s, exc, 1 H), 3.82 (s, 3 H), 5.26 (br s, 1 H), 7.14 (d, $J = 2$ Hz, H), 7.26 (d, $J = 2$ Hz, 1 H); ^{13}C NMR (acetone-d_6) δ 22.1, 22.6, 25.2, 28.0, 32.2, 56.0, 102.1, 112.3, 120.8, 1233.7, 136.0, 136.1, 156.6, 158.8, 191.9. Anal. ($C_{15}H_{18}O_4$) C, H.

4-Methoxyspiro[benzofuran-2(3*H*)-cyclohexane]-6-carboxylic Acid (5a). Amberlyst 15 (1.0 g) was added in one portion to a solution of **11a** (70 mg, 0.27 mmol) in benzene (10 mL), and the mixture was stirred at room temperature until no starting material was observed in TLC. The suspension was filtered, and the filtrate was concentrated to afford 64 mg (91%) of **5a** as off-white crystals: mp 203−205 °C (ether/acetone); IR (cm^{-1}) 2930, 1675, 1600, 1425, 1330, 1275, 1220, 1125, 1035, 950, 780, 735; ^1H NMR (acetone-d_6) δ 1.63 (br m, 10 H), 2.91 (s, 2 H), 3.87 (s, 3 H), 7.00 (s, 1 H), 7.13 (s, 1 H); ^{13}C NMR (acetone-d_6) δ 23.6, 25.7, 37.8, 39.1, 55.9, 90.5, 104.8, 105.0, 119.8, 132.8, 157.3, 160.9, 167.7; HRMS, exact mass for $C_{15}H_{18}O_4$, calcd m/z 262.1205, obsd m/z 262.1205. Anal. ($C_{15}H_{18}O_4$) C, H.

4-Methoxyspiro[benzofuran-2(3*H*)-cyclohexane]-6-carboxaldehyde (12). Intermediate **10** (50 mg, 0.2 mmol) was cyclized using the above procedure for **5a** to afford 48 mg (96%) of **12** as a solid: mp 88−89 °C (ether/hexanes); IR (cm^{-1}) 2940, 2850, 1690, 1600, 1325, 1220, 1130, 1110, 1035, 835, 685; ^1H NMR δ 1.70 (br m, 10 H), 2.82 (s, 2 H), 3.88 (s, 3 H), 6.89 (s, 1 H), 6.93 (s, 1 H), 9.87 (s, 1 H); ^{13}C NMR δ 23.0, 25.1, 37.2, 38.5, 55.6, 90.4, 102.8, 105.6, 121.3, 138.2, 156.9, 160.5, 191.8. Anal. ($C_{15}H_{18}O_3$) C, H.

4-Hydroxyspiro[benzofuran-2(3*H*)-cyclohexane]-6-carboxaldehyde (13). Compound **12** was demethylated as described for **9**. Thus, *t*-BuSH (0.28 mL, 2.5 mmol) in HMPA (2 mL), 2.4 M *n*-BuLi (1 mL, 2.4 mmol), and **12** (201 mg, 0.82 mmol) in HMPA (5 mL) were reacted to give a crude product that was purified by column chromatography to afford 165 mg (87%) of **13** as fine needles: mp 115−116 °C (ether/hexanes); IR (cm^{-1}) 3250, 2930, 1665, 1585, 1305, 1280, 1250, 1205, 1175, 840, 805, 740; ^1H NMR δ 1.3−1.9 (br m, 10 H), 2.97 (s, 2 H), 6.85 (s, 1 H), 6.90 (s, 1 H), 9.80 (s, 1 H); ^{13}C NMR δ 23.0, 25.0, 37.2, 38.1, 90.4, 104.3, 108.6, 120.3, 138.0, 153.2, 161.2, 192.2; HRMS, exact mass for $C_{14}H_{16}O_3$, calcd m/z 232.1099, obsd m/z 232.1010.

4-Hydroxyspiro[benzofuran-2(3*H*)-cyclohexane]-6-carboxylic Acid (5b). Compound **13** was oxidized as described for **10**. Thus, **13** (48 mg, 0.21 mmol) was oxidized in a suspension of Ag_2O (120 mg, 0.52 mmol) in 5% aqueous NaOH to afford 49 mg (96%) of **5b** as a light-brown powder: mp 223−225 °C (acetone/ether); IR (cm^{-1}) 3300, 2940, 1720, 1610, 1435, 1225, 1200, 1060, 965; ^1H NMR (acetone-d_6) δ 1.75 (br m, 10 H), 2.94 (s, 2 H), 3.73 (br s, exc, 1 H), 6.87 (s, 1 H), 7.08 (s, 1 H); ^{13}C NMR (acetone-d_6) δ 23.6, 25.7, 37.8, 39.0, 90.1, 103.0, 109.9, 118.6, 132.4, 154.9, 161.3, 167.9; HRMS, exact mass for $C_{14}H_{16}O_4$, calcd m/z 248.1049, obsd m/z 248.1050. Anal. ($C_{14}H_{16}O_4$) C, H.

4-Methoxy-7-(*N*-methyl-*N*′-piperazinyl)carboxyspiro[benzofuran-2(3*H*)-cyclohexane] Hydrochloride (5j). To a solution of **5c** (450 mg, 1.72 mmol) in dry $CHCl_3$ (7 mL), $SOCl_2$ (1 mL, 13.7 mmol) was added, and the reaction mixture was stirred at 50−55 °C for 2.5 h. The mixture was concentrated in vacuo, the crude acid chloride (ca. 480 mg) was dissolved in dry toluene (10 mL) and stirred at room temperature, and *N*-methylpiperazine (1 mL, 9 mmol) was added in one portion. The reaction mixture was stirred for an additional 1.5 h and concentrated in vacuo, and the residue was shaken with 25% aqueous K_2CO_3 (15 mL) and extracted with ether (2 × 50 mL). The combined ether extracts were dried (K_2CO_3), concentrated in vacuo, and chromatographed (silica/1% v/v TEA in toluene) to provide 55 mg (93%) of the free base of **5f** as a light-yellow oil: IR (neat, cm^{-1}) 2935, 2856, 2792, 1623, 1430, 1286, 1097, 1002, 917, 761; ^1H NMR δ 1.5−1.42 (br m, 4 H), 1.78−1.66 (br m, 6 H), 2.31 (s, 3 H), 2.35 (m, 2 H), 2.45 (br m, 2 H), 2.89 (s, 2 H), 3.41 (br m, 2 H), 3.78 (br m, 1 H), 3.83 (s, 3 H), 6.43 (d, 1 H, $J = 9.5$ Hz), 7.16 (d, 1 H, $J = 9.5$ Hz), 7.24 (br m, 1 H); ^{13}C NMR δ 23.15, 25.07, 37.36, 37.86, 46.13, 55.44, 90.47, 103.03, 111.89, 113.33, 125.29, 128.22, 129.03, 129.77, 156.14, 157.75, 167.36; MS (thermospray) m/z 345 (MH^+).

Hydrochloride. The free base of **5j** (530 mg, 1.54 mmol) was dissolved in dry ether (10 mL) and acidified with an ethanolic solution of HCl to pH 3.0, after which the precipitate was filtrated, washed with ether, and dried in vacuo to afford 456 mg of **5j** as a colorless solid: mp 255−256 °C (EtOH/ether). Anal. ($C_{20}H_{28}N_2O_3 \cdot HCl$) C, H, N, Cl.

4-Methoxyspiro[benzofuran-2(3*H*)-cyclohexane]-7-hydroxamic Acid (5k). The acid chloride was prepared from **5c** (1.31 g, 5 mmol) in dry $CHCl_3$ (15 mL) and $SOCl_2$ (2 mL, 27.4 mmol) as above, and the mixture was stirred at 50−55 °C for 2.5 h. The mixture was concentrated in vacuo, and the crude acid chloride (ca. 1.4 g) was dissolved in dry toluene (12 mL). The mixture was added dropwise to a stirred and cooled (to 0 °C) solution prepared from $NH_2OH \cdot H_2SO_4$ (2.0 g, 12.2 mmol) and 85% KOH (2.5 g, 38 mmol) at 0−5 °C. The reaction mixture was stirred at room temperature for 1 h, poured onto a mixture of ice/water (40 mL), and extracted with ether (2 × 20 mL). Drying ($MgSO_4$) and concentration in vacuo afforded 0.8 g of the crude product, which was purified by chromatography to provide 210 mg (15.1%) of **5k** as a light-pink solid: mp 165−167 °C (95% EtOH); IR (cm^{-1}) 3370, 3118, 2929, 2858, 1648, 1463, 1284, 1093, 1043, 917, 765; ^1H NMR δ 1.95−1.4 (br m, 10 H), 2.91 (s, 2H), 3.85 (s, 3 H), 6.49 (d, 1 H, $J = 9.5$ Hz), 7.86 (d, 1 H, $J = 9.5$ Hz), 9.93 (br s, ex 1 H); ^{13}C NMR δ 23.22, 24.88, 37.18, 37.76, 55.57, 92.90, 103.66, 106.19, 113.54, 130.50, 157.29, 159.48, 163.74; MS (thermospray) m/z 278 (MH^+), Anal. ($C_{15}H_{19}NO_4$) C, H, N.

4-(1′-Cyclohexenyl)methyl-3,5-dimethoxybenzyl Alcohol (14). The procedure used for coupling was as described for **9**. Thus, 2.2 M *n*-BuLi (32 mL, 70.4 mmol) was added to a cooled solution of **6** (5.0 g, 29.7 mmol) and TMEDA (10.5 mL, 69.7 mmol) in dry THF (300 mL) at 0 °C. After 2 h of stirring at room temperature, the mixture was cooled to −78 °C, CuI (7.0 g, 36.7 mmol) was added, and the suspension was warmed to −45 °C and stirred at that temperature over a 90 min period. The reaction mixture was recooled again to −78 °C, and **8** (6.8 g, 38.8 mmol) was added. The mixture was stirred at room temperature for 24 h. The mixture was worked up as for **9**, and the crude product was purified by chromatography to give 6.0 g (77%) of **14** as white needles: mp 61−63 °C (hexanes); IR (cm^{-1}) 3290, 2930, 1590, 1460, 1425, 1210, 1140, 1120; ^1H NMR δ 1.56 (br m, 4 H), 1.93 (br m, 4 H), 2.62 (br s, 1 H, exc), 3.26 (br s, 2 H), 3.79 (s, 6 H), 4.59 (s, 2 H), 5.20 (br s, 1 H), 6.54 (s, 2 H); ^{13}C NMR δ 22.5, 23.1, 25.3, 28.8, 30.6, 55.8, 65.7, 102.5, 116.6, 119.9, 136.3, 139.8, 158.5. Anal. ($C_{16}H_{22}O_3$) C, H.

6-(1′-Cyclohexenyl)methyl-5,7-dimethoxy-1(3*H*)-isobenzofuranone (15). A solution of 2.0 M *n*-BuLi in hexanes (4.2 mL, 8.4 mmol) was added to a stirred solution of **14** (1.0 g, 3.82 mmol) and TMEDA (0.69 mL, 4.58 mmol) in hexanes (50 mL) at 0 °C under a nitrogen stream. The reaction mixture was warmed slowly to room temperature, stirred for an additional 1.5 h, and recooled to −78 °C, after which dry carbon dioxide was bubbled for 1 h at −78 °C and over 1 h at room temperature. A solution of 2 N NaOH (25 mL) was added, the unreacted material was extracted with ether (50 mL), the aqueous phase was acidified with 6 N HCl, and the reaction product was extracted with ether (4 × 50 mL). Drying ($MgSO_4$) and concentration of the organic phase afforded 727 mg (66%) of **15** as a solid: mp 103−104 °C (hexanes/ether); IR (cm^{-1}) 3000−2820, 1740, 1600, 1460, 1420, 1340, 1240, 1200, 1090, 1015 and 940; ^1H NMR δ 1.48−2.00 (m, 8 H), 3.30 (s, 2 H), 3.88 (s, 3 H), 4.03 (s, 3 H), 5.19 (br s, 1 H), 5.20 (s, 2 H), 6.64 (s, 1 H); ^{13}C NMR δ 22.5, 23.1, 25.3, 28.9, 31.0, 56.2, 62.6, 68.8, 98.7, 109.8, 121.0, 123.0, 136.2, 148.8, 158.4, 164.3. Anal. ($C_{17}H_{20}O_4$) C, H.

6-(1′-Cyclohexenyl)methyl-7-hydroxy-5-methoxy-1(3*H*)-isobenzofuranone (16). Compound **15** was demethylated to **16** according to the procedure described for **9.** From **15** (470 mg, 1.63 mmol) in dry HMPA (10 mL), *t*-BuSLi was prepared in situ from *t*-BuSH (0.56 mL, 10 mmol) in HMPA (4 mL) and 2.4 N *n*-BuLi (2.0 mL, 4.8 mmol) after the mixture was stirred at room temperature until no more starting material was left as monitored by TLC. The reaction product was extracted from the aqueous acidic solution with ethyl acetate (4 × 40 mL). Drying ($MgSO_4$) and concentration of the organic phase afforded 332 mg (74%) of **16** as a white solid: mp 150−151.5 °C (hexanes/ether); ^{1}H NMR δ 1.48−2.00 (m, 8 H), 3.27 (s, 2 H), 3.88 (s, 3 H), 5.2 (s, 2 H), 6.5 (s, 1 H), 7.71 (s, 1 H); ^{13}C NMR δ 22.4, 23.0, 25.2, 28.7, 30.2, 56.2, 70.4, 96.1, 104.2, 115.5, 120.9, 135.4, 146.2, 155.0, 165.2, and 172.8.

Cyclohexanespiro-2′-tetrahydrofuran[4′,5′-g]-5-methoxy-1(3*H*)-isobenzofuranone (5c). Compound **16** was cyclized to **5c** according to the procedure described above for **5a**. Thus, **16** (40 mg, 0.146 mmol) and Amberlyst 15 (290 mg) in dry CH_2Cl_2 (3 mL) were stirred overnight at room temperature to afford 38 mg (95%) of **5c** as a solid: mp 132−134 °C (hexanes/ether); IR (cm^{-1}) 2980−2820, 1740, 1610, 1440, 1330, 1280, 1260, 1235, 1205, 1145, 1080, 1010, 930, and 780; ^{1}H NMR δ 1.35−2.00 (m, 10 H), 2.89 (s, 2 H), 3.91 (s, 3 H), 5.20 (s, 2 H), 6.46 (s, 1 H); ^{13}C NMR δ 23.1, 25.0, 37.1, 55.9, 69.8, 93.7, 96.0, 102.0, 114.8, 150.2, 157.8, 162.1, 169.6. Anal. ($C_{16}H_{18}O_4$) C, H.

4-Methoxy-6-formylspiro[benzofuran-2(3*H*)-cyclohexane]-7-carboxylic Acid (5d). A solution of **5c** (211 mg, 0.77 mmol) in THF (4 mL) was added to a stirred solution of 1.0 M $(Bu)_4NF$ in THF (1 mL, 1 mmol) and 10 N NaOH (6 mL, 60 mmol). Solid $KMnO_4$ in small portions was added to the reaction mixture until no more starting material was observed by TLC monitoring. The excess of $KMnO_4$ was destroyed by the dropwise addition of a saturated solution of Na_2SO_3. The reaction mixture was acidified with 6 N H_2SO_4 and extracted with ethyl acetate (4 × 50 mL). The organic phase was dried ($MgSO_4$), concentrated, and purified by chromatography to yield 120 mg (53%) of **5d** as a colorless solid: mp 128−130 °C (hexanes/ether); IR (cm^{-1}) 2950, 2850, 1830, 1770, 1625, 1455, 1340, 1270, 1210, 1145, 990, 985, 745; ^{1}H NMR δ 1.35−1.95 (m, 10 H), 2.86 (s, 2 H), 3.91 (s, 3 H), 5.90 (br s, 1 H), 6.53 (s, 1 H), 6.61 (s, 1 H); ^{13}C NMR δ 23.1, 25.0, 37.1, 55.9, 69.8, 93.7, 96.0, 102.0, 114.8, 150.2, 157.8, 162.1, 169.6. Anal. ($C_{16}H_{18}O_5$) C, H.

6,7-Diformyl-4-methoxyspiro[benzofuran-2(3*H*)-cyclohexane] (18). A solution of 2.1 M *n*-BuLi in hexanes (11 mL, 23.2 mmol) was added to a solution of *N*,*N*,*N*′-trimethylethylenediamine (3 mL, 23.5 mmol) in dry THF (28 mL) at −20 °C under a nitrogen stream. After 30 min, compound **17** (5.5 g, 22.3 mmol) in THF (19 mL) was added dropwise, followed 30 min later by 2.1 M *n*-BuLi in hexanes (31 mL, 65.1 mmol) and DMF (10.3 mL, 132 mmol). The reaction mixture was kept at −20°C for 24 h. The reaction products were partitioned between ether (4 × 200 mL) and brine (200 mL), and chromatography of the extracts gave 2.1 g (34.3%) of **18** pure enough for the additional steps of the synthesis (mp over 100 °C). The analytical sample was successively recrystallized to afford mp 119−126 °C (ether/hexanes); IR (cm^{-1}) 3000−2840, 1670, 1600, 1470, 1425, 1390, 1320, 1280, 1260, 1210, 1130, 1030, 890, 850, 770, 700, 620; ^{1}H NMR δ 1.40−1.93 (s, 10 H), 2.93 (s, 2 H), 3.94 (s, 3 H), 7.04 (s, 1 H), 10.35 (s, 1 H), 10.70 (s, 1 H); ^{13}C NMR δ 22.9, 24.9, 37.2, 37.7, 56.0, 93.0, 103.6, 113.7, 120.2, 138.7, 160.3, 164.7, 188.6, 192.6. Anal. ($C_{16}H_{18}O_4$) C, H.

6,7-Dicarboxyl-4-methoxyspiro[benzofuran-2(3*H*)-cyclohexane] (5e). To a solution of **18** (153 mg, 0.56 mmol) in EtOH (5 mL) was added a solution of $AgNO_3$ (222 mg, 1.3 mmol) in distilled water (1 mL), followed by 1 N KOH (3 mL, 3 mmol). The system, shielded from light, was stirred overnight at room temperature and filtered, and the solid residue was washed with water. The combined aqueous phases were washed with ether, the aqueous phase was acidified with 2 N H_2SO_4 and extracted with ether (3 × 25 mL), and the combined organic phases were dried and chromatographed to afford 156 mg (91%) of **5e** as a colorless solid: mp 188−190 °C (acetone); IR (cm^{-1}) 3500−2400, 3000−2850, 1700, 1610, 1410, 1330, 1290, 1130, 1040, 1000, 930, 855, 750, 660; ^{1}H NMR (acetone-d_6) δ 1.40−1.90 (m, 10 H), 2.93 (s, 2 H), 3.91 (s, 3 H), 6.95 (s, 2 H); ^{13}C NMR (acetone-d_6) δ 23.5, 25.6, 37.6, 38.7, 56.1, 91.7, 105.1, 111.6, 118.8, 133.0, 157.8, 158.8, 167.2, 186.1. Anal. ($C_{16}H_{18}O_6$) C, H.

6-Carboxyl-7-formyl-4-methoxyspiro[benzofuran-2(3*H*)-cyclohexane] (5f). Compound **18** (2.1 g, 8 mmol) in THF (6 mL) was treated with $AgNO_3$ (2.27 g, 13.3 mmol) in water (4 mL) and 4 N KOH (8 mL, 32 mmol) as described for **5e**. After 2 h of stirring at room temperature and workup as for **5e**, the final acidic ether extracts concentrated in vacuo afforded 423 mg (14.3%) of crude product (MS thermospray indicated a molecular ion m/z 291 = MH$^+$), which after successive recrystallization provided 153 mg (6.7%) of **5f** as a colorless solid: mp 132−134 °C (ether/hexanes); IR (cm^{-1}) 3440, 3000−2840, 1740, 1630, 1450, 1345, 1290, 1240, 1150, 1105, 1010, 910, 860, 770, 690; ^{1}H NMR (DMSO-d_6) δ 1.30−1.52 (m, 4 H), 1.62−1.81 (m, 6 H), 2.91 (s, 2 H), 3.87 (s, 3 H), 6.55 (br s, 1 H), 6.85 (s, 1 H), 7.95 (br s, 1 H); ^{13}C NMR (DMSO-d_6) δ 22.52, 24.27, 36.57, 37.56, 55.86, 91.90, 96.33, 98.34, 120.60, 121.48, 154.03, 158.28, 168.42; EIMS (m/z, relative intensity) 290 (M$^+$, 89), 289 (72), 272 (100), 271 (88), 244 (65), 215 (78), 192 (98), 190 (65), 165 (93), 164 (82), 79 (89). Anal. ($C_{16}H_{18}O_5$) C, H.

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