Synthesis and Solution Dynamics of Agrobactin A

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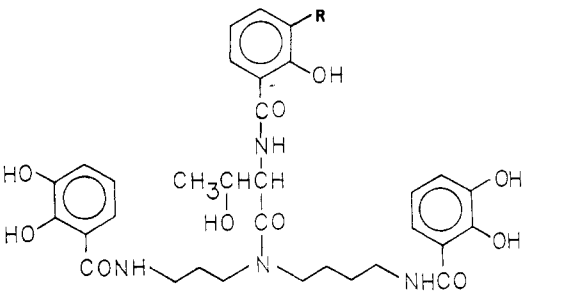
A high-yield synthesis of agrobactin A and its symmetrical homologue, homoagrobactin A, is described. The key to the synthesis is an "inside-out" approach that couples the terminal 2,3-dihydroxybenzoyl groups of agrobactin A to the spermidine backbone in the final step. This scheme makes the cumbersome protection and deprotection of the catechol groups unnecessary. As proof of structure, agrobactin A is also synthesized by previous methods. High-field ^1H NMR spectroscopy is employed to evaluate the coalescence temperatures and activation energies controlling conformer interconversion of these polyamides, and, finally, the role of steric and hydrogen-bonding factors in solution dynamics is discussed.

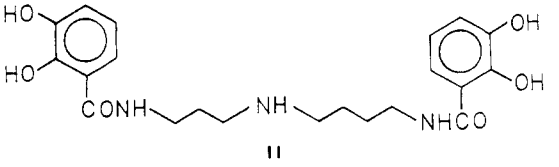
Since the isolation and identification of N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine (compound II, Chart I) and N^4-[N-(2-hydroxybenzoyl)threonyl-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine (parabactin A, Ib) from *Paracoccus dentrificans* by Tait in 1975,[1] there has been considerable interest in the synthesis of spermidine-derived iron chelators.[2-7] In earlier reports we described the facile high-yield synthesis of compound II and a variety of its homologues,[8,9] and more recently we have reported on the first total synthesis of parabactin (IIIb),[10] the threonyl-cyclized form of parabactin A. Initially, parabactin A was thought to be the microorganism's parent siderophore as during the original isolation; unbeknownst to the investigator, parabactin's oxazoline ring was hydrolyzed to the corresponding threonyl compound.[11] An analogous system has now been isolated from *Agrobacterium tumifaciens* B_6 cultures,[12] agrobactin A (Ia) and agrobactin (IIIa). Like parabactin, agrobactin is a spermidine-derived siderophore; however, it contains a third 2,3-dihydroxybenzoyl moiety in place of parabactin's 2-hydroxybenzoyl ring. As with the parabactin/parabactin A system, agrobactin A (Ia) is the open oxazoline-hydrolyzed form of agrobactin (IIIa).

While agrobactin A has been recently synthesized by Neilands by reacting spermidine with the bulky reagent (2,3-dibenzoyloxy)benzoyl chloride, followed by attachment of the N-[(2,3-dibenzyloxy)benzoyl]threonyl "centerpiece" and removal of the benzoyloxy protection groups,[13] the yields reported for the condensation steps and the subsequent removal of the benzoyloxy protecting groups make this synthesis unrealistic for scaleups. We now report a short high-yield synthesis of agrobactin A and a symmetrical homologue, homoagrobactin A. Unlike previous syntheses of catecholamides by us and other groups that attached the "centerpiece" in the final stages of the synthesis, the synthesis of agrobactin A reported here describes an "inside-out" approach, attaching the catechols

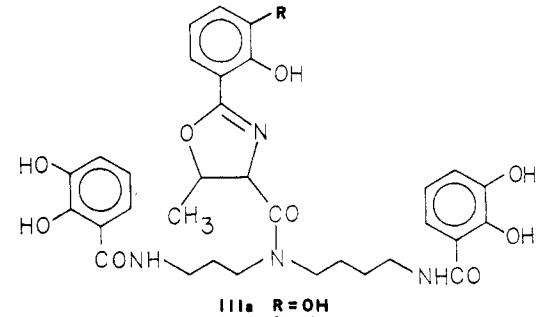
Chart I



Ia R=OH
Ib R=H



II



IIIa R=OH
IIIb R=H

in the final step. Because of this feature, no catechol-protecting groups are necessary since mild condensation conditions are employed. As a proof of structure, we also report the synthesis of agrobactin A via our earlier methods,[10] which build the molecule from the "outside in".

High-field ^1H NMR studies have indicated that both agrobactin and parabactin exist in a set of interconverting isomers (conformers) of unequal population. However, it remains to be proven whether or not this is true of agrobactin A and parabactin A. Furthermore, although an activation energy for conformer interconversion has been reported for agrobactin, no such information exists for the open homologue, agrobactin A; nor is there any information as to the origin of the observed conformers, i.e., the contribution of steric and hydrogen-bonding factors toward the activation energy controlling conformer interconversion. Accordingly, with the synthesis of agrobactin A at

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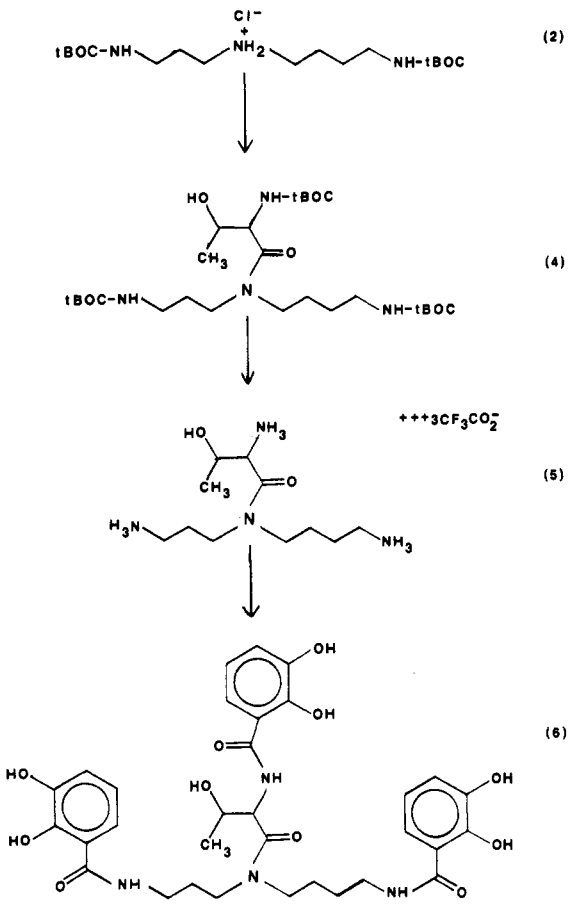
(13) Neilands, J. B.; Peterson, T.; Falk, K. E.; Leong, S. A.; Klein, M. D. *J. Am. Chem. Soc.* **1980**, *102*, 7715.

0022-3263/83/1948-3432$01.50/0

Scheme I



hand, further high-field ^{1}H NMR and coalescence studies of agrobactin A and its homologues were undertaken.

Synthesis

The "inside-out" synthesis of agrobactin A begins with the reagent N^1,N^8-bis(*tert*-butoxycarbonyl)spermidine **2**. Originally developed by us as a reagent to selectively generate N^4-modified spermidine derivatives for the delivery of antineoplastics to leukemia cells,[14,15] it is stable and easily accessible in high yields. Furthermore, both the homo- and norspermidine homologues are also available, allowing for the synthesis of the homo- and noragrobactin A homologues.

The synthesis of these reagents is easily effected by reacting the appropriate secondary *N*-benzyl polyamine with [[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile, followed by debenzylation to afford the bis(terminally *t*-Boc-protected) polyamine in 90% overall yield.[14] Agrobactin A and its homospermidine homologue are subsequently synthesized in three high-yield steps from this point, as illustrated for agrobactin A in Scheme I.

The procedure first calls for reacting the succinimido ester of *t*-Boc-threonine, previously generated by reacting (*tert*-butoxycarbonyl)threonine (**1**), *N*-hydroxysuccinimide, and dicyclohexylcarbodiimide (DCC) in THF, with a solution of N^1,N^8-bis(*tert*-butoxycarbonyl)spermidine hydrochloride (**2**) or its homospermidine analogue and triethylamine in aqueous CH_3CN for 48 h. The condensation proceeds smoothly and cleanly to afford the *t*-Boc triamide (**4**) in 90% crude yield. This product can be easily purified via silica gel chromatography, eluting with $CHCl_3$/EtOAc (1:1); however, further purification was found to be unnecessary as the impurities can be removed after the next step. Hence, **4** is deprotected by brief exposure to trifluoroacetic acid. The residue is dissolved in water, and the impurities from the preceding condensation are extracted into $CHCl_3$ prior to lyophilization of the aqueous layer to afford N^4-threonylspermidine **5** or N^5-threonylhomospermidine **9** as the trifluoroacetate salt in 80% overall yield from *N*-(*tert*-butoxycarbonyl)threonine.

The final and most crucial step of the synthesis is the addition of the three 2,3-dihydroxybenzoyl moieties to the N^4-threonyl triamine. Previous studies suggested it would be necessary first to protect the catechol groups of 2,3-dihydroxybenzoic acid prior to condensation. However, because of the known acid-catalyzed N to O migration of acyl groups fixed to threonine's nitrogen,[16,17] only protecting groups capable of being removed under neutral or basic conditions could be considered. Under acid conditions threonine's *N*-acyl carbonyl becomes more electrophilic, promoting intramolecular transacylation, resulting in the formation of the threonyl ester and the amine salt. The migration can be reversed, in some cases, under basic conditions.[16] During our initial synthesis of compound II we had developed the reagent 2,3-diacetoxybenzoyl chloride, which could efficiently acylate spermidine's primary amines, and the acetoxy protecting groups could then be cleanly removed by treatment with methanolic sodium methoxide.[18]

When triamine **5** was reacted with 3 equiv of 2,3-diacetoxybenzoyl chloride, the acetoxy protecting groups removed under basic conditions, and the products worked up in weak acid, two products were repeatedly obtained in low yields. The major product was determined to be the N to O migrated ester of agrobactin A, as indicated by its 300-MHz ^{1}H NMR spectra characterized by the chemical shifts of the α-, β-, and γ-methyl protons of δ 5.2, 5.5, and 1.4, respectively. This migration was likely the result of protonating the catechols during acidic workup. Even when cold pH 6.5 phosphate buffer was employed to protonate the catechols, substantial migration still occurred. Furthermore, the minor product although identical on TLC with agrobactin A, demonstrated a ^{1}H NMR spectrum with the α- and β-methines shifted 0.2 ppm upfield to those values previously reported. In addition, obvious differences in the aromatic region were also noted. In light of these problems, this method was abandoned.

Alternatively, we considered the possibility of attaching the 2,3-dihydroxybenzoyl groups directly to the N^4-threonyl triamine without catechol protecting groups. Recently, Van Brussel and Van Sumere have shown it possible to generate the succimido-active esters of a number of mono- and dihydroxybenzoic acids in the presence of the unprotected phenols.[19] This approach, besides being the most direct, would generate agrobactin A under essentially neutral conditions. This is desirable as it would eliminate the use of acid to protonate the catechols, reducing or eliminating the amount of migrated ester formed.

Therefore, *N*-hydroxysuccinimido-2,3-dihydroxybenzoate (**3**) was prepared by reacting 2,3-dihydroxybenzoic acid and *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide to afford **3** upon recrystalli-

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zation from methanol/H_2O. The final step of the synthesis was successfully accomplished by reacting 3 equiv of the active ester **3** with N^4-threonylspermidine in aqueous THF for 48 h. Thin-layer chromatography of the products indicate that the reaction proceeds with little or no formation of the undesired migrated ester. The reaction mixture was concentrated and the crude agrobactin A easily purified by chromatography on Sephadex LH-20 to afford agrobactin A (**6**) in 75% yield. The symmetrical homologue of agrobactin A, N^5-[*N*-(2,3-dihydroxybenzoyl)]-N^1,N^9-bis(2,3-dihydroxybenzoyl)homospermidine (**10**) was prepared by reacting **3** with N^5-threonylspermidine **9** and purified in a similar manner. Additionally, the hexamethyl derivative of agrobactin *A*, N^4-[*N*-(2,3-dimethoxybenzoyl)threonyl]-N^1,N^8-bis(2,3-dimethoxybenzoyl)spermidine (**11**) was also synthesized by using these procedures. The succimide active ester of 2,3-dimethoxybenzoic acid was generated as previously for **3** and reacted with triamine **5** to produce hexamethyl agrobactin A (**11**) in 95% yield. The synthesis of **11**, in addition to providing us with a necessary derivative for our ^{1}H NMR studies, illustrates that the number of compounds that can be generated by this method are limited only by the acylating or alkylating agent employed.

Finally, as proof of structure, agrobactin A was synthesized by an alternate route with the versatile reagent N^1,N^8-bis(2,3-dimethoxybenzoyl)spermidine.[8] As previously described in our synthesis of parabactin,[10] N^1,N^8-bis(2,3-dimethoxybenzoyl)spermidine was condensed with *N*-(carbobenzyloxy)threonine, again by using the coupling agents dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccimide, to produce N^4-[*N*-(carbobenzyloxy)threonyl]-N^1,N^8-bis[2,3(dimethoxybenzoyl)spermidine. The Cbz protecting group is then removed by hydrogenolysis in methanolic HCl over $PdCl_2$ catalyst, followed by the removal of the methoxy groups with BBr_3 to afford N^4-threonyl-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine. The final product was produced by reacting the succinimido ester of 2,3-dihydroxybenzoic acid (**3**) with N^4-threonyl-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine. Although this second method also represents an effective means of generating agrobactin A, the preceding inside–out synthesis is preferred preparatively as it contains one less step and requires only one chromatographic separation instead of three.

Experimental Section

Materials. All reagents, with the exception of [[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile (BOC-ON, Sigma Chemical) were purchased from Aldrich Chemical Co. and, unless noted, were used without further purification. Na_2SO_4 was used as the drying agent. Melting points were taken on a Fischer-Johns apparatus and are uncorrected. Routine ^{1}H NMR spectra were recorded on a Varian T-60 spectrometer and prepared in $DCCl_3$ or Me_2SO-d_6 with chemical shifts given in parts per million (δ) from an internal Me_4Si standard. The infrared spectra were recorded on a Beckman 4210 spectrophotometer. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA.

***N*-(*tert*-Butoxycarbonyl)threonine (1)** was prepared by standard literature procedure by reacting D,L-threonine with [[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile:[20] mp 121–122 °C (Et_2O/petroleum ether) (lit. 122–123 °C).

N^1,N^8-Bis(*tert*-butoxycarbonyl)spermidine·HCl (2) and N^1,N^9-bis(*tert*-butoxycarbonyl)homospermidine·HCl (7) were prepared and purified as previously described.[14]

Succinimido 2,3-Dihydroxybenzoate (3). To a solution of 2,3-dihydroxybenzoic acid (1.16 g, 7.53 mmol) and *N*-hydroxysuccinimide (1.04 g, 9.04 mmol) in dry dioxane (30 mL) was added DCC (1.89 g, 9.16 mmol) in dioxane (20 mL) under N_2. After 16 h the mixture was filtered and the DCC washed with dioxane (15 mL). The solvent was evaporated in vacuo and the residue crystallized from MeOH/H_2O to yield 1.8 g (95%) of product as tan crystals. mp 168–171 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.93 (4 H), 6.52–7.36 (3 H), 9.82 (2 H).

Anal. Calcd for $C_{11}H_9NO_6$: C, 52.60; H, 3.61; N, 5.58. Found: C, 52.68; H, 3.66; N, 5.52.

N^4-[*N*-(*tert*-Butoxycarbonyl)threonyl]-N^1,N^8-bis(*tert*-butoxycarbonyl)spermidine (4). A solution of DCC (680 mg, 3.3 mmol) in 20 mL of THF was slowly added to a solution of **1** (660 mg, 3.0 mmol) and *N*-hydroxysuccimide (380 mg, 3.3 mmol) in 30 mL of THF. After 18 h, the DCU precipitates were filtered and washed with fresh THF, and and the filtrate was evaporated. The residue was dissolved in 30 mL of CH_3CN and slowly added to a solution of **2** (1.26 g, 3.3 mmol) and Et_3N (470 μL, 3.5 mmol) in 5% aqueous CH_3CN (50 mL). After being stirred for 36 h at room temperature, the reaction mixture was then concentrated and the residue taken up in 100 mL of EtOAc, washed with H_2O (2 × 20 mL), 3% HCl (3 × 20 mL), and H_2O (2 × 20 mL), dried, and concentrated to afford 1.5 g (91%) of the desired product.

An analytical sample was obtained by chromatography on silica gel, eluting with EtOAc/$CHCl_3$ (1:1): ^{1}H NMR ($CDCl_3$) δ 1.20 (d, 3 H), 1.44 (s, 27 H), 1.75 (m, 6 H), 2.80–3.58 (overlapping multiplets, 8 H), 4.04 (m, 1 H), 4.38 (d, 1 H), 4.8–5.5 (br, 4 H); IR ($CHCl_3$) 3000 (m), 1715 (s), 1180 (s), 770 (s) cm^{-1}.

Anal. Calcd for $C_{28}H_{50}N_4O_8$: C, 57.12; H, 9.22; N, 10.45. Found: C, 57.25; H, 9.24; N, 10.17.

N^4-Threonylspermidine Trifluoroacetate (5). Trifluoroacetic acid (25 mL) was slowly added to a cooled flask containing **4** (650 mg, 1.2 mmol) and the resulting solution was stirred for 25 min while warming to room temperature. The solvent was then quickly evaporated, and the residue dissolved in MeOH (25 mL) and evaporated (twice). The product was then dissolved in 50 mL of H_2O and washed with cold $CHCl_3$ (3 × 10 mL), and the aqueous layer was lyophilized to afford 690 mg (97%) as a light tan hygroscopic solid.

An analytical sample was prepared by chromatography on Sephadex LH-20, eluting with 20% MeOH/EtOAc: ^{1}H NMR (Me_2SO-d_6) δ 1.15 (d, 3 H), 1.36–2.08 (m, 6 H), 2.80–3.32 (m, 8 H), 4.52 (m, 3 H), 7.94 (m, 9 H); IR (KBr) 1685 (s), 1190 (s) cm^{-1}.

Anal. Calcd for $C_{17}H_{29}F_9N_4O_8$: C, 34.70; H, 4.97; N, 9.52. Found: C, 34.50; H, 5.03; N, 9.43.

N^4-[*N*-(2,3-Dihydroxybenzoyl)threonyl]-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine (Agrobactin A) (6). A solution of 2,3-dihydroxybenzoyl *O*-succinimido ester (**3**) (350 mg, 1.4 mmol) in 15 mL of THF was slowly added dropwise to a solution of **5** (275 mg, 0.46 mmol) and Et_3N (210 mL, 1.5 mmol) in 5% aqueous THF (40 mL) under N_2. After 36 h, the solvent was evaporated to dryness, and the residue was preabsorbed on Sephadex LH-20 and eluted with an ethanol/benzene gradient (5–25% v/v) to yield 225 mg (75%) of the desired product. The spectral characteristics were identical with those reported in the literature.[13]

Anal. Calcd for $C_{32}H_{38}N_4O_{11}\cdot H_2O$: C, 57.13; H, 5.99; N, 8.33. Found: 57.10; H, 6.09; N, 8.26.

N^5-[*N*-(*tert*-Butoxycarbonyl)threonyl]-N^1,N^9-bis(*tert*-butoxycarbonyl)homospermidine (8) was prepared and purified as described for **4** by reacting **7** in place of **2**: 89% yield; ^{1}H NMR ($CDCl_3$) δ 1.18 (d, 3 H), 1.48 (s, 27 H), 1.40–1.71 (m, 8 H), 3.02–3.56 (m, 8 H), 4.03 (m, 1 H), 4.36 (d, 1 H), 4.72–5.48 (br, 4 H); IR ($CHCl_3$) 3010 (m), 1715 (s), 1175 (s), 770 (s) cm^{-1}.

Anal. Calcd for $C_{27}H_{52}N_4O_8$: C, 57.83; H, 9.35; N, 9.99. Found: C, 57.56; H, 9.41; N, 9.92.

N^5-Threonylhomospermidine Trifluoroacetate (9) was prepared and purified as described for **5**: 98% yield; ^{1}H NMR (Me_2SO-d_6) δ 1.18 (d, 3 H), 1.36–1.99 (m, 8 H), 2.82–3.37 (m, 8 H), 4.54 (m, 3 H), 7.97 (m, 9 H).

Anal. Calcd for $C_{18}H_{31}F_9N_4O_8$: C, 35.89; H, 5.19; N, 9.30. Found: C, 35.54; N, 5.27; N, 9.14.

N^5-[*N*-(2,3-Dihydroxybenzoyl)threonyl]-N^1,N^9-bis(2,3-dihydroxybenzoyl)homospermidine (homoagrobactin A) (10) was prepared and purified as described for **6**: 72% yield; NMR (See Table I).

Anal. Calcd for $C_{33}H_{40}N_4O_{11}$: C, 59.27; H, 6.03; N, 9.57; Found: C, 58.91; H, 6.19; N, 9.37.

(20) (a) Itoh, M.; Hagiwara, D.; Kamiya, T. *Tetrahedron Lett.* **1975**, 4393. (b) The D,L stereochemistry is assumed in all subsequent products.

Table I. 300 MHz ^{1}H Chemical Shifts and Coupling Constants of Polyamides (23 °C)

threonine	agrobactin A ($CDCl_3/Me_2SO$-d_6)	homoagrobactin A ($CDCl_3/Me_2SO$-d_6)	4 ($CDCl_3$)	8 ($CDCl_3$)
α	5.02, $J_{\alpha NH}$ = 8.4 Hz	5.03, $J_{\alpha NH}$ = 8.4 Hz	4.39, $J_{\alpha NH}$ = 9.6 Hz	4.39, $J_{\alpha NH}$ = 9.6 Hz
β	4.18, $J_{\alpha\beta}$ = 2.7 Hz	4.21, $J_{\alpha\beta}$ = 3.0 Hz	4.02	4.02
γ	1.20, $J_{\beta\gamma}$ = 6.3 Hz	1.19, $J_{\beta\gamma}$ = 6.6 Hz	1.18, $J_{\beta\gamma}$ = 6.1 Hz	1.17, $J_{\beta\gamma}$ = 6.3 Hz
OH	~4.7	~4.7	5.2	4.2
NH	8.09	8.1	5.47	5.48
t-Bu			1.45	1.44
CCH_2C	1.5–2.1	1.5–1.85	1.4–1.9	1.36–1.7
NCH_2C	3.15–3.75	3.15–3.65	2.95–3.6	3.12, 3.30, 3.50
aromatics	6.70, 7.00, 7.22	6.68, 6.98, 7.22		
NH	8.03, 8.24	8.0, 8.15	4.70, 4.97	4.70, 5.00
phenols	12.0, 13.0	12.0, 13.0		

Table II. Coalescence Temperatures and Activation Energies of Agrobactin A and Homologues in Me_2SO-d_6

	T_c, °C	E_a, kcal/mol^{-1}
precursor 4	18[a]	15.2 ± 0.15
agrobactin A (6)	74	18.2 ± 0.15
agrobactin A-OMe (11)	>130	>21
agrobactin[b]	~75	~18

[a] Determined in DMF-d_7. [b] From ref 13.

N^4-[*N*-(2,3-Dimethoxybenzoyl)threonyl]-N^1,N^8-bis(2,3-dimethoxybenzoyl)spermidine (Hexamethyl Agrobactin A) (11). A solution of DCC (450 mg, 2.2 mmol) in THF (15 mL) was slowly added to a solution of 2,3-dimethoxybenzoic acid (400 mg, 2.0 mmol) and *N*-hydroxysuccimide (255 mg, 2.2 mmol) in THF (30 mL). The reaction was allowed to stir at room temperature for 18 h at which time the DCU precipitates were filtered off and washed with THF. The filtrate was then concentrated to approximately 20 mL and added to a solution of **5** (350 mg, 0.60 mmol) and Et_3N (90 μL, 0.65 mmol) in 5% aqueous CH_3CN (30 mL). After 48 h, the solvent was evaporated and the product chromatographed on silica gel, eluting with 10% MeOH/$CHCl_3$ to yield 400 mg (91%) as a white hygroscopic solid: ^{1}H NMR ($CDCl_3$) δ 1.16 (d, 3 H), 1.58 (m, 6 H), 3.42 (m, 8 H), 3.56 (s, 18 H), 4.0–4.6 (m, 3 H), 6.62–7.7 (m, 9 H), 7.74–8.4 (m, 3 H).

Anal. Calcd for $C_{38}H_{50}N_4O_{11}$: C, 61.78; H, 6.82; N, 7.58. Found: C, 61.84; H, 6.71; N, 7.47.

Agrobactin A (via Scheme II). A mixture of D,L-N^4-threonyl-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine–hydrogen bromide[10] (100 mg, 0.17 mmol) and triethylamine (24 μL, 0.17 mmol) in dry DMF (20 mL) was cooled to 0 °C under N_2. A solution of *N*-hydroxysuccinimido-2,3-dihydroxybenzoate (**3**) (43 mg, 0.17 mmol) in DMF (20 mL) was added dropwise over 15 min. After 12 h, the solvent was removed in vacuo and the residue chromatoghraphed on LH-20 (10→30% EtOH/benzene) to afford 82 mg (75%) of product as a white solid. The TLC and 300-MHz ^{1}H NMR spectral characteristics of this product were identical with that of agrobactin A in Scheme I.

^{1}H NMR Spectroscopy. High-resolution FT ^{1}H NMR spectra were recorded on a Nicolet NT-300 spectrometer equipped with a Nicolet 1280 computer. Samples were run at room temperature (23 ± 1 °C) unless otherwise noted. Generally, when $CDCl_3$ or $CDCl_3/Me_2SO$-d_6 was employed as the solvent, 1–4 mg of sample was dissolved in approximately 500 μL of solvent. Chemical shift and coupling constant values, when obtainable, are given in Table I in the solvents indicated. All chemical shifts are in ppm downfield from an internal Me_4Si standard. Samples for the temperature-dependence studies were prepared by dissolving 5–10 mg of sample in 500 μL of Me_2SO-d_6 or DMF-d_7 as indicated in Table II. Activation energies were measured by observing the coalescence temperature of the resulting γ-methyl singlets produced upon irradiation of the adjacent β-methine. The coalescence temperatures were measured both on heating and cooling cycles, and activation energies were subsequently calculated by using the rate equations as described by Gutowsky and Cheng.[21] Additionally, the results were found to be in good agreement with those calculated from a total line-shape analysis using a program contained in the NT-300 software for unequally populated two-site exchange. The results indicated that the experimental δν's were within 1 Hz of the simulated values. Therefore, by assignment of a maximum error of ±1.0 °C in T_c and an error of ±1.0 Hz in measurement of the δν, the range of error in determining the E_a is ±0.15 kcal/mol.

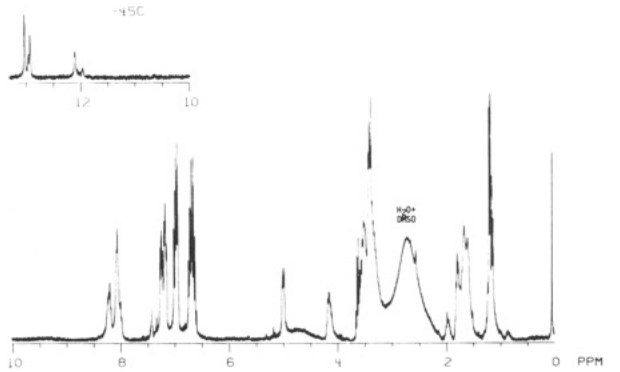

Figure 1. 300-MHz ^{1}H NMR spectrum of agrobactin A in $CDCl_3/Me_2SO$-d_6 (10:1) at 23 °C. The downfield phenolic region at −45 °C is shown in the insert.

Results and Discussion

The 300-MHz ^{1}H NMR spectrum of agrobactin A in $CDCl_3/Me_2SO$-d_6 (10:1) at 23 °C is shown in Figure 1. This solvent system was employed for the purpose of comparison with earlier published spectra of agrobactin A as well with agrobactin and parabactin. The choice of solvent is critical as the chemical shifts of these compounds are extremely sensitive to changes in solvent. The δ values observed are identical with the chemical shifts previously reported for agrobactin A,[13] and the overall appearance of the spectra is similar to the previously published ^{1}H NMR spectra of agrobactin[13] and parabactin,[10] noting, however, the differences in the α, β, and γ threonine resonances between the open and closed forms.

For purposes of analysis, the spectra can be divided into two regions, Figure 1; the upfield portion above δ 6.00 containing the resonances from the spermidine backbone and the threonine residue and the downfield portion containing the aromatic, phenolic, and amido protons. The high-field end of the spectrum is characterized by a pair of doublets centered at δ 1.20, which integrates to three protons. These protons are assigned to the γ-methyl of threonine, each doublet equally coupled to the β-methine ($J_{\beta\gamma}$ = 6.3 Hz) as confirmed by decoupling experiments. The envelope between δ 1.5 and 2.1 integrates to six protons and originates from the three internal methylene groups of the spermidine backbone, while the four external methylene groups adjacent to the amides are responsible for the eight-proton envelope observed from δ 3.15 to 3.75.

(21) Gutowsky, H. S.; Cheng, H. N. *J. Chem. Phys.* **1975**, *63*, 2439.

The final set of peaks in the upfield region of the spectra include an extensively split multiplet at δ 4.18 and an apparent doublet of doublets at δ 5.02, both integrating to one proton each. These have been assigned as the β- and α-methines, respectively, by the appropriate decoupling experiments. Additionally, a broad hump is observed at about δ 4.7, resulting from the threonine hydroxyl which disappears on exchange with D_2O. Furthermore, as expected, the hydroxyl's intensity and location will vary with concentration and temperature.

The downfield portion of the spectra consists of the aromatic protons, amido protons, and the phenolic hydroxyls. The aromatic region lies between δ 6.6 and 7.4 and integrates to nine protons. Their assigment, although similar to the aromatics of N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine reported earlier,[22] is further complicated by the third 2,3-dihydroxybenzoyl moiety. The upfield overlapping multiplets centered at δ 6.70 integrate to three protons and can be assigned to the meta protons. Downfield to this are four lines centered at δ 7.00, which correspond to the three para protons. Finally, the ortho protons appear the furthest dowfield as two apparent triplets centered at δ 7.19 and 7.25, the upfield triplet integrating to two protons vs. one for the downfield triplet. The NH protons make up the next group of signals downfield from the aromatics; those resulting from spermidine's terminal amides are located at δ 8.03 and 8.24 while the threonyl N–H is located between them at δ 8.09. The phenolic protons lie the furthest downfield and, like the threonine hydroxyl, undergo exchange at 23 °C. This exchange results in broad, undefined signals whose locations are again dependent upon such factors as temperature and the amount of water present. At –45 °C, however, the rate of exchange is considerably slowed, resulting in sharper signals at approximately δ 12.0 and 13.0 as shown in the inset in Figure 1.

Of particular interest, however, is the duplicity of signals originating from the threonyl moiety. The threonine coupling pattern, in the absence of any conformational effects, represents a simple spin system. The α-methine should be split once by the amide, resulting in a doublet with a $J \approx 6$–9 Hz. This doublet should then be further split by the β-methine to produce a doublet of doublets. The β-methine is coupled to the three γ-methyl protons and should result in a quartet, which is split again by the α-methine to produce eight lines, which would be expected to give rise to a complex multiplet. Finally, the γ-methyl should be split principally by the single β-methine proton, resulting in a doublet with an expected coupling on the order of 5.5–6.5 Hz, as long-range α–γ coupling is likely to be small.

Inspection of Figures 2b and 3b reveals that although the predicted coupling patterns do exist, they exist in duplicate. For example, the γ-methyl group exists as a pair of doublets instead of a single doublet, Figure 2b. These doublets are observed in a ratio of approximately 2:1 in $CDCl_3/Me_2SO$-d_6 and are both equally coupled to the β-methine (J = 6.3 Hz). Likewise, the β-methine also exhibits an additional set of signals. This is more clearly seen when the γ-methyl is decoupled, Figure 3b. Again, in the absence of any conformational effects, irradiation of the γ-methyl group should result in the β-methine collapsing to a doublet arising from the coupling with the α-methine (J = 2.6 Hz). However, in agrobactin A, irradiation of the γ-methyl results in a pair of doublets centered at δ 4.2, Figure 3b, also in approximately a 2:1 ratio.

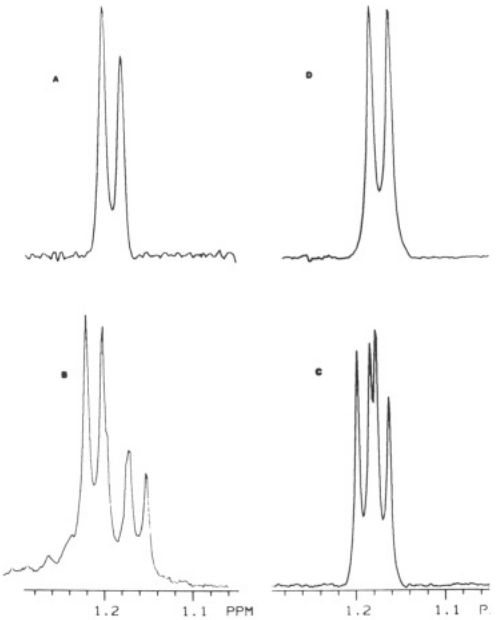

Figure 2. 300-MHz ^{1}H NMR spectra of γ-methyl region in solvents indicated: (a) homoagrobactin A (**10**) ($CDCl_3/Me_2SO$-d_6); (b) agrobactin A (**6**) ($CDCl_3/Me_2SO$-d_6); (c) N^4-[N-*tert*-butoxycarbonyl)threonyl]-N^1,N^8-bis(*tert*-butoxycarbonyl)spermidine (**4**) ($CDCl_3$); (d) N^5-[N(*tert*-butoxycarbonyl)threonyl]-N^1,N^9-bis(*tert*-butoxycarbonyl)homospermidine (**8**) ($CDCl_3$).

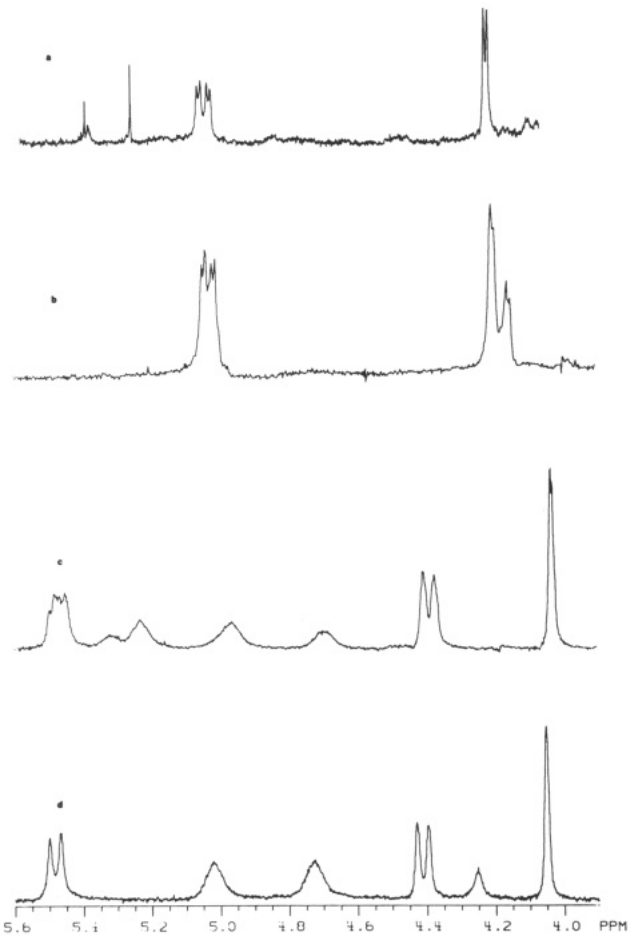

Figure 3. 300-MHz ^{1}H NMR spectra of the α- and β-methine regions of (a) homoagrobactin A, (b) agrobactin A, (c) precursor **4**, and (d) precursor **8** in the solvents indicated in Figure 2. The γ-methyl protons are decoupled to simplify the spectra.

(22) Bergeron, R. J.; Burton, P. S.; Kline, J. J.; McGovern, K. A. *J. Org. Chem.* **1981**, *46*, 3712.
(23) Eng-Wilmot, D. L.; van der Helm, D. *J. Am. Chem. Soc.* **1980**, *102*, 7719.

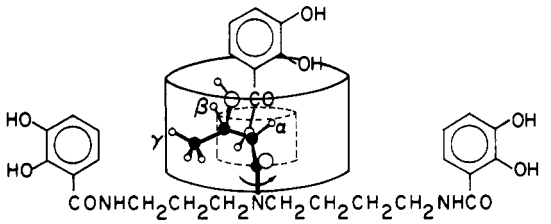

Figure 4. Diagram illustrating the approximate sweep volumes of the α- and γ-protons of agrobactin A on rotation about the central amide.

Surprisingly enough, the α-methine, however, does not show an additional set of lines, giving rise to the expected doublet of doublets as one might anticipate, i.e., split once by the amide (J = 8.4 Hz) and once by the β-methine (J = 2.6 Hz). This observation differs from what is observed for parabactin or agrobactin, where duplicate signals are observed for the α-methine.

The ^{1}H NMR data are consistent with the threonine substituent existing in at least two distinct magnetic environments. Because the β-methine and γ-methyl protons are located further out on the threonyl side chain, i.e., closer to the terminal 2,3-dihydroxybenzoyl groups than the α-methine, on rotation about the central amide bond they see more significant changes in magnetic environment. The α-methine, having a more "internal" location, moves through a relatively smaller area upon rotation and at greater distances from the aromatic rings. Therefore, it sees little or no change in its environment, resulting in only one observable signal (Figure 4). The inner and outer cylinders represent the approximate sweep volumes of the α-methine and γ-methyl group, respectively. The γ-methyl group and β-methine can lie in close proximity to the terminal aromatic rings and can easily be influenced by anisotropic effects. However, since these effects decrease rapidly with increasing distances, the α-methine is likely to be too distant from the aromatic rings to be affected by their anisotropy.

Furthermore, if the cylinders are cut in half by a plane passing through the central nitrogen perpendicular to the spermidine backbone, it can easily be seen that corresponding points in either half are at different intramolecular distances from the aromatic rings due to the asymmetry of the spermidine chain. Accordingly, these points will also experience different magnetic fields. Therefore, a proton existing in a conformation lying in the left half will likely result in a different signal as compared to its counterpart that lies in the right half of the cylinder. Although this model does not consider all of the possible orientations of the spermidine backbone and aromatic rings, a recent X-ray crystallographic study of agrobactin[23] does suggest a conformation with a nearly linear spermidine backbone that is similar to that depicted in the model.

On the basis of this model, a symmetrical analogue should equalize the intramolecular distances on either half, eliminating the duplicity of signals observed for the β- and γ-protons of agrobactin A.

Neilands has also attributed the appearance of the ^{1}H NMR spectrum of agrobactin A to the asymmetrical nature of the spermidine chain and, in an attempt to resolve the problem, synthesized a symmetrical analogue to agrobactin A, containing a glycine residue instead of the threonine residue.[13] As we have noted, the α-methine of agrobactin A does not appear to "see" a difference in conformation; hence, a model lacking the threonine side chain may not be the appropriate model to verify the symmetry (or asymmetry) of the polyamine backbone in the ^{1}H NMR spectrum.

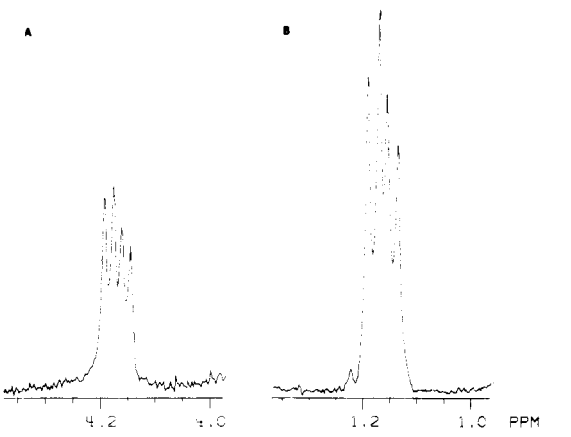

Figure 5. 300-MHz ^{1}H NMR spectra of agrobactin A in DMF-d_7: (a) γ-methyl-decoupled β-methine region; (b) γ-methyl region.

Accordingly, for verification of the glycine work, the symmetrical analogue of agrobacin A incorporating homospermidine as the backbone was synthesized. As expected, the symmetrical homoagrobactin A resulted in a simplified spectrum. The γ-methyl of homoagrobactin A now exhibits only a single doublet, Figure 2a, as does its partially decoupled β-methine located at δ 4.2, Figure 3a. The appearance of the α-methine, δ 5.0, is similar in both homoagrobactin A and agrobactin A, Figures 3a and 3b, respectively, although the doublet structure of the latter appears to be broader, indicating that, perhaps, minor a conformational effect is being felt by the α-methine in agrobactin A. Similar results are observed in the 300-MHz ^{1}H NMR spectrum of the asymmetrical *tert*-butoxycarbonyl precursor **4** and its symmetrical homologue **8**. The γ-methyl protons of **4** are observed as two overlapping doublets centered at δ 1.18, Figure 2c. However, in the symmetrical precursor **8**, only one doublet is observed for the γ-methyl, δ 1.17, Figure 2d. Additionally, the threonyl NH (δ 5.48) is simplified in **8** and is observed as a doublet, Figure 3d, as compared to a doublet of doublets for **4**, Figure 3c. On the other hand, the α- and β-methines (δ 4.41 and 4.04, respectively) of **4** experience little change in their magnetic environment as a result of conformation, Figure 3c.

It is interesting that the β-methine of **4** lacks the duplicity of its counterpart in agrobactin A; however, this might be explained by the differences in anisotropy generated by the *tert*-butoxycarbonyl and 2,3-dihydroxybenzoyl groups. Additionally, the conformation of the threonine residue may be different between agrobactin A and **4**. This is supported by the observation that no coupling is seen between the α- and β-methines of **4** compared to the J = 2.6 Hz value in agrobactin A (Table I), indicative of a difference in dihedral angle between the α- and β-methines in agrobactin A and **4**.

It was mentioned earlier that the chemical shifts of agrobactin A and its related siderophores are extremely sensitive to the ^{1}H NMR solvent employed. Similarly, the conformer population of agrobactin A was found to be affected by solvent. As previously illustrated by agrobactin A's γ-methyl and β-methine signals in Figures 2b and 3b, the conformers exist in approximately a 2 to 1 ratio in $CDCl_3/Me_2SO$-d_6 (10:1). After switching to a more polar hydrogen-bond acceptor solvent such as Me_2SO or DMF, the conformer population appears to even out, approaching a 1:1 ratio as illustrated in Figure 5 for DMF. This solvent dependency suggests that hydrogen bonding may play a role in determining the conformer population. It is suspected that Me_2SO or DMF is interacting intermolecularly with agrobactin A, reducing the intramolecular interactions

responsible for the conformer preference seen in the less polar $CDCl_3$.

Since agrobactin A, agrobactin, and N^4-[*N*-*tert*-butoxycarbonyl)threonyl]-N^1,N^8-bis(*tert*-butoxycarbonyl)-spermidine (**4**) all exhibit duplicity in their ^1H NMR spectra, the energy of activation (E_a) for the interconversion between the conformers can be estimated by measuring the coalescence temperature (T_c) of these compounds. A qualitative comparison of the estimated E_a's might therefore provide additional information regarding the factors affecting conformation in these siderophores. Although the two γ-methyl signals corresponding to the two polyamide conformers do not quite represent an equally populated spin system, the E_a for their interconversion can be estimated by using methods developed for equally populated, noncoupled spin systems.[21] It is generally acknowledged that computer total-line-shape analysis should be employed for highly accurate values of E_a; however, highly accurate values are not required for the qualitative comparisons necessary in these studies. In this regard, the relative differences in E_a's between the compounds are more important than their absolute values.

Of all the barriers involved in controlling the interconversion between the conformers in these polyamides, hydrogen bonding and steric factors are likely to be the dominant ones. The question is how to determine the contribution of each of these components to the interconversion. The answer is to carefully remove or introduce hydrogen bonding and/or steric interactions in the systems of interest and then evaluate the effect on E_a. In this study we have chosen to consider the E_a of agrobactin, agrobactin A, the precursor **4**, and methylated agrobactin A (**11**). The structural differences and similarities between these four compounds should be sufficient to help assign the roles of steric and hydrogen-bonding factors controlling the conformer populations of the spermidine siderophores.

The coalescence temperatures of agrobactin A, hexamethyl agrobactin (**11**), and precursor **4** were all determined in Me_2SO-d_6 as described in the Experimental Section and are listed in Table II. Irradiation of the β-methine of agrobactin A, for example, results in the collapse of the γ-methyl resonance into two lines at room temperature. Upon heating, the two lines at first broaden gradually, then more rapidly as the coalescence temperature is approached. Finally, the lines are observed to coalesce at approximately 74 °C, and with a further increase in temperature, the line width of the resultant single line now decreases as its intensity increases.

The observed T_c of 74 °C for agrobactin corresponds to an activation energy of approximately 18.2 ± 0.15 kcal/mol. This is quite similar to the values reported for agrobactin by Neilands and was unexpected in view of the difference between the relatively rigid oxazoline ring vs. the more flexible open agrobactin A. A second unexpected result was the high coalescence temperature of the methylated derivative **11**. It was initially anticipated that methylation of the catechols would lower the coalescence temperature of the various conformers by destroying the stabilizing hydrogen-bonding network. On the contrary, **11** coalesced at a much higher temperature than agrobactin A. In fact, the T_c of **11** exceeded the operating limit of our probe, preventing the precise measurements of **11**'s E_a. At 130 °C, the highest temperature that could be measured, the decoupled γ-methyl signals of **11** were nearing coalescence. The E_a of **11** was, therefore, estimated, based on a T_c of at least 130 °C and a $\delta\nu$ of 9.4 Hz, to be greater than 21 kcal/mol. Since the E_a varies directly with temperature, the further increase in temperature above 130 °C can only increase the E_a. Therefore, 21 kcal/mol represents the minimal E_a for **11**. This large increase in E_a for **11** over agrobactin A can be explained by an increase in steric interactions that are preventing interconversion between conformers. It does not, however, necessarily mean that steric factors are more important than hydrogen bonding, as the solvent, Me_2SO-d_6, used in the measurements is certainly capable of competing for intramolecular hydrogen bonds. This implies that the initial measurement of E_a for agrobactin A made in Me_2SO is largely a measurement of steric control of the conformer population, as intramolecular hydrogen bonding is likely minimized by intermolecular competition with Me_2SO.

The coalescence temperature of the tri-*t*-Boc precursor **4** further supports the idea that Me_2SO prevents intramolecular hydrogen bonding in the systems studied. As shown in Figure 2c, the γ-methyl of **4** exists as an overlapping set of doublets in $CDCl_3$ at room temperature. In Me_2SO-d_6, however, even at room temperature only one doublet is observed. Therefore, the change from the less polar $CDCl_3$ to Me_2SO-d_6 is sufficient alone to allow rapid interconversion between conformers of **4**.

The high freezing point of Me_2SO precluded its further use in determining the T_c value of **4**; therefore, an alternate solvent was chosen. However, in order to make a comparison of the E_a's between **4** in an alternate solvent to those of agrobactin and its homologues in Me_2SO-d_6, it is important that the solvent itself does not change the E_a significantly. DMF has a similar dielectric to Me_2SO but a much lower freezing point necessary for the measurement, making it an appropriate choice. To determine if DMF would have any effect on the E_a, the T_c and E_a of agrobactin A was subsequently measured in DMF. No significant difference was noted between the E_a and T_c of agrobactin A determined in DMF vs. Me_2SO, suggesting the data of **4** in DMF can be compared to those in Me_2SO.

The ^1H NMR spectrum of **4** in DMF-d_7 at room temperature also lacked the duplicity seen in $CDCl_3$. However, upon cooling, the decoupled γ-methyl signal broadened and eventually displayed two signals that coalesced at 18 °C, corresponding to a E_a of 15.2 ± 0.15 kcal/mol. This low E_a observed for **4** as compared to agrobactin A was anticipated at least in direction, if not in magnitude. The reason for this may not lie in a difference in overall size between the *tert*-butyl vs. the 2,3-dihydroxybenzoyl group, as molecular models suggest that planar 2,3-dihydroxybenzoyl group is only slightly bulkier. However, it may indicate that a small role is played by catechol hydrogen bonding in the conformer populations of agrobactin A vs. the precursor **4**. Alternately, the ether linkage present in the *tert*-butoxycarbonyl group may provide the *tert*-butyl group an additional degree of freedom, making it easier for it to avoid the centerpiece as it rotates.

The activation energies of the polyamides are all consistent with the energy required for the cis–trans isomerization of the amide bond. The relatively large (~3 kcal/mol) differences in E_a between agrobactin A, **4**, and **11** suggests that the terminal acyl substituents play a significant role in the intramolecular interactions of those polyamides. Furthermore, the coalescence data suggest that in solvents such as Me_2SO, which can complete for intramolecular hydrogen bonds, steric factors primarily influence interconversion between conformers in the systems studied. It further implies that the intramolecular hydrogen bonding in agrobactin A is not unusually strong. It is certain, however, that hydrogen bonding must play a role in conformer population in nonpolar solvents. This was illustrated by the effect of solvent upon duplicity of

^{1}H NMR signals in agrobactin A and, most dramatically, in the precursor 4 in which Me_2SO-d_6 spectra lacked any sign of the conformers present in $CDCl_3$ at 23 °C. This effect on the ^{1}H NMR of 4, on changing between Me_2SO and $CDCl_3$, is of additional interest in view of the fact that the *tert*-butoxy group is not able to form as many hydrogen bonds as the 2,3-dihydroxybenzoyl group. This suggests that an even greater solvent effect may be experienced by agrobactin A and its related siderophores, producing much higher T_c and E_a values in nonpolar solvents for these compounds. However, due to agrobactin A's poor solubility in all but the most polar solvents, a comprehensive determination of T_c's in other solvents is essentially not possible.

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Registry No. 1, 86748-77-8; 2, 83392-11-4; 3, 86748-78-9; 4, 86748-79-0; 5, 86748-81-4; 6, 86783-92-8; 7, 83392-12-5; 8, 86748-82-5; 9, 86748-84-7; 10, 86748-85-8; 11, 86748-86-9; D,L-threonine, 80-68-2; 2,3-dihydroxybenzoic acid, 303-38-8; succimimidyl *N*-*tert*-butyloxycarbonyl-D,L-threoninate, 86783-93-9; 2,3-dimethoxybenzoic acid, 1521-38-6; D,L-N^4-threonyl-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine hydrobromide, 86783-94-0.

Phthalide Annulation: The Synthesis of Kalafungin, Pachybasin, and Chrysophanol

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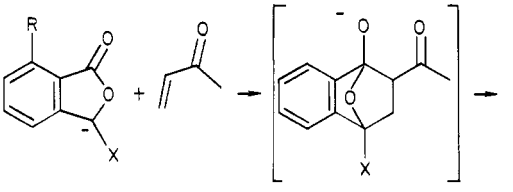
The anions of 3-cyano- or 3-(phenylthio)phthalide react with Michael acceptors to afford functionalized naphthydroquinones in good yield. The cyano and phenylthio groups function both as activating groups and as leaving groups. An alternative involves the use of protected *o*-(carboxymethyl)cyanohydrins. The use of phthalide anions in synthesis is exemplified by total syntheses of kalafungin, pachybasin, and chrysophanol. This methodolgy consititutes a direct and regiospecific approach to polycyclic systems.

Introduction

Michael addition followed by base-induced cyclization consitutes a versatile method for the construction of carbocyclic rings. Several variants of this concept have been developed.[1] A common feature of these reactions is that four carbons of the newly formed cyclohexenone ring are supplied by the Michael acceptor. A generally useful Michael addition sequence in which four carbons of the newly formed cyclohexane ring originate from the Michael donor would represent a valuable contribution to synthetic methodology.

In this paper we report the use of functionalized phthalides and ortho-substituted benzoates to effect an annulation reaction. The hydroquinone products of this reaction can be readily oxidized to quinones. Quinones are important subunits in physiologically active molecules such as daunomycin[2] (1), chrysophanol[3] (2), and kalafungin[4] (3). One advantage of this methodology over sequential Friedel–Crafts acylations is that the regiochemistry of the product is unambiguous. In Friedel–Crafts reactions, certain rearrangements such as the Hayashi rearrangement can produce mixtures of isomeric products. This method should complement the Diels–Alder reaction as a useful method for the construction of polycyclic quinones.

Conceptually related cyclizations have recently been described. Hauser and co-workers have investigated the chemistry of anions of sulfonylphthalides and have reported a clever synthesis of kidamycinone.[5] Sammes[6] has studied the reactions of phthalide anions with Michael acceptors. The yields of naphthols obtained by his procedure were modest. VanLeusen and co-workers[7] have



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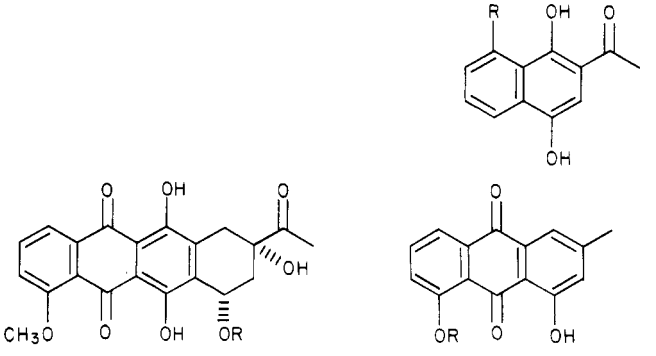
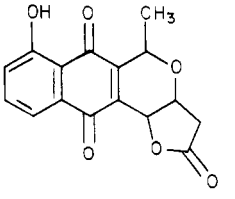


1, R = daunosamine

2, chrysophanol, R = H



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