at 405 nm using a Titretek Multiscan Mk310. The amount of Dnp-containing peptide (X) per well was calculated using

$$X = [37.53 \times (\text{absorbance}) - 0.38] \text{ nmol}$$

Peptide Appraisal. Analytical HPLC was performed on a Waters Associates liquid chromatography system comprised of two 510 pumps, a WISP 710B autosampler, a Model 440 UV detector (254 nm) with an extended wavelength module (214 nm), and a DEC Professional work station. A 5-μm Merck Lichrosphere 100 RP-18 (250 × 4 mm i.d.) column was used. Gradient elution from A to B was carried out in 5–20 min with the following buffer system: A, 0.1% TFA in water, and B, 0.1% TFA in water/acetonitrile (40:60 v/v).

Amino acid analysis was carried out using PITC derivatives[20] as previously reported.[13] Positive ion FAB mass spectra were recorded by Dr. D. P. Kelly and Mr. B. Shirriffs, University of Melbourne. Samples were suspended in a thioglycerol matrix and ionized by bombardment with xenon ions.

Acknowledgment. We wish to thank Wayne Sampson, David Stanton, Brian Sutherland, and Michael FitzGerald for their skilled and enthusiastic technical support.

Biosynthesis of Sarubicin A. Synthesis and Incorporation of 6-Hydroxy[$^{13}CO^{15}NH_2$]anthranilamide[1]

Steven J. Gould* and Rodney L. Eisenberg

Department of Chemistry, Oregon State University, Corvallis, Oregon 97331-4003

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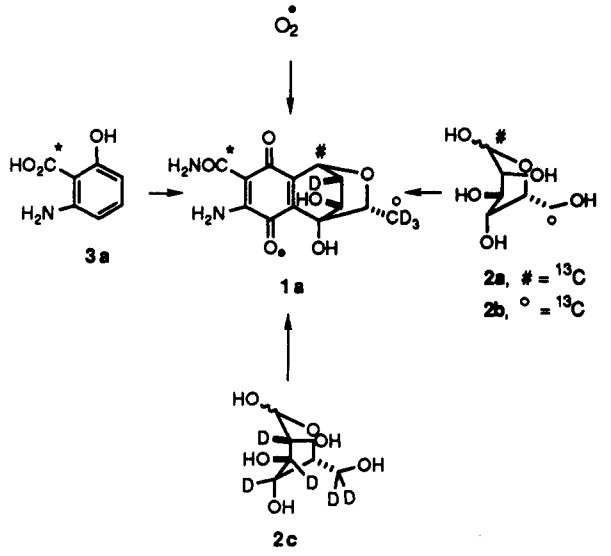
We have previously demonstrated that 6-hydroxyanthranilic acid (**3**) is specifically incorporated into sarubicin A (**1**) by *Streptomyces helicus*. 6-Hydroxyanthranilamide (**4**) has now been synthesized in five steps from *m*-anisidine in a manner that allowed efficient introduction of isotope labels to prepare [$^{13}CO^{15}NH_2$]-**4a**. A new synthesis of **3** from *m*-anisidine has also been developed. **4a** was fed to *S. helicus*, and a 1.29% incorporation into **1b** was obtained. Examination of the ^{13}C NMR spectrum of **1b** revealed predominantly intact incorporation, with a minor amount of **4a** (0.13%) first undergoing in vivo hydrolysis to the corresponding acid. Thus, carboxamide formation is the next step in the biosynthesis of **1**.

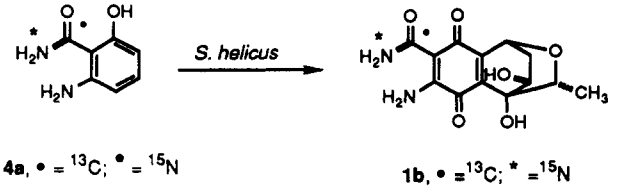
Sarubicin A (**1**), a quinone antibiotic produced by *Streptomyces helicus*, was first characterized in 1980[2] and its absolute stereochemistry established in 1983.[3] Two total syntheses have since been reported.[4] Early, unpublished results by a group at The Upjohn Company established the labeling pattern from [1-^{13}C]-D-glucose (**2a**) and [6-^{13}C]-D-glucose (**2b**), which indicated intact incorporation of glucose into the tetrahydropyran portion of **1**[5] and suggested a derivation of the quinonoid portion of **1** from the shikimate pathway.[6]

We subsequently established that [$^{13}COOH$]-6-hydroxyanthranilic acid (**3a**) labeled the carboxamide carbon of **1** and that the C-4 quinone carbonyl oxygen, but not that at C-1, was derived from $^{18}O_2$.[7] 6-Hydroxyanthranilic acid had not previously been known as a natural product. Results obtained from the incorporation of [2,3,4,6,6-$^2H_5$]-D-glucose (**2c**) showed retention of deuterium at the C-7_{cis} and methyl hydrogens in a 1:3 ratio, indicating replacement of the C-2 hydroxyl by hydrogen (retention of configuration) and migration of the C-4 deuterium to C-6 of glucose.[8] These results are summarized in Scheme I with the composite structure **1a**.

Scheme I



Scheme II



It was anticipated that subsequent key steps in the biosynthetic pathway leading to **1** would be formation of a *C*-glycoside, introduction of the C-6 phenol, cyclization, and generation of the carboxamide. In order to test whether carboxamide formation might be the first of these, 6-hydroxyanthranilamide labeled with both ^{13}C and ^{15}N

(1) Studies on Nitrogen Metabolism Using ^{13}C NMR Spectroscopy. 6. Part 5: Prabhakaran, P. C.; Woo, N.-T.; Yorgey, P.; Gould, S. J. *Tetrahedron Lett.* **1986**, *27*, 3815–3818.

(2) (a) Tresselt, D.; Eckhardt, K.; Ihn, W.; Radics, L.; Reinhardt, G. *Tetrahedron* **1981**, *37*, 1961–1965. (b) Reinhardt, G.; Bradler, G.; Eckardt, K.; Tresselt, D.; Ihn, W. *J. Antibiot.* **1980**, *33*, 787–790. (c) Slechta, L.; Chidester, C.; Rensser, F. *J. Antibiot.* **1980**, *33*, 919–923.

(3) Eckardt, K.; Tresselt, D.; Ihn, W.; Kajtar, M.; Angyan, J.; Radics, L.; Hollosi, M. *J. Antibiot.* **1983**, *36*, 976–979.

(4) (a) Takeuchi, T.; Sudani, M.; Yoshii, E. *J. Org. Chem.* **1983**, *48*, 4151–4152. (b) Semmelhack, M. F.; Appapillai, Y.; Sato, T. *J. Am. Chem. Soc.* **1985**, *107*, 4577–4579.

(5) Slechta, L., private communication.

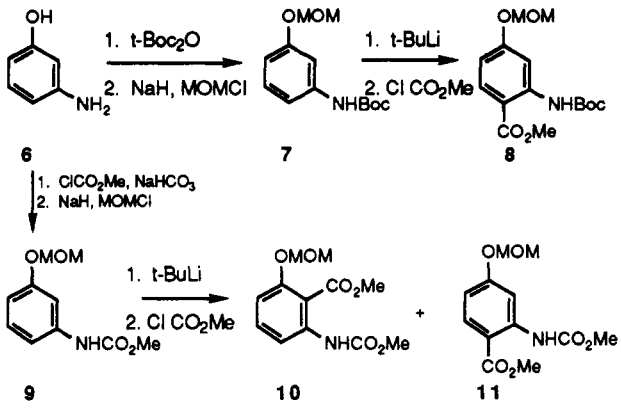
(6) Haslam, E. *The Shikimate Pathway*; Halsted Press: New York, 1974.

(7) Hillis, L. R.; Gould, S. J. *J. Am. Chem. Soc.* **1985**, *107*, 4593–4594.

(8) Gould, S. J.; Tann, C.-H.; Prabhakaran, P. C.; Hillis, L. R. *Bioorg. Chem.* **1988**, *16*, 258–271.

0022-3263/91/1956-6666$02.50/0

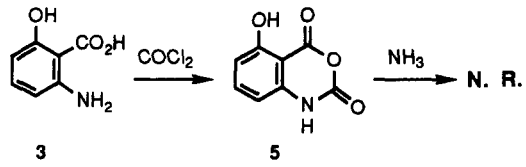
Scheme III



at the carboxamide (**4a**) was required. Intact incorporation of **4a** would yield a ^{13}C–^{15}N heteronuclear spin coupling in the derived antibiotic, which should be detectable by ^{13}C NMR spectroscopy.[9] We now report the synthesis of **4** by a route that allowed efficient introduction of the necessary labels, the preparation of **4a**, and the biosynthetic incorporation of **4a** into **1** (Scheme II).

Results and Discussion

Synthesis. Since we had previously developed a synthesis of **3** that allowed introduction of a ^{13}C label at the carboxyl carbon,[10] initial efforts focused on attempts to directly convert **3** to **4**. Isatoic anhydrides had been shown to react with ammonium hydroxide as the solvent to afford amides in good yield,[11] but these conditions would have been prohibitively expensive utilizing ^{15}N. Anhydride **5** was prepared from **3** and phosgene and then treated with ammonia in various solvents and at various temperatures[12] in an attempt to develop an economical procedure. However, in each case either no reaction occurred or decomposition resulted.



In order to introduce the nitrogen label at an earlier stage of our previous synthesis of **3**,[10] the route was now altered to provide a more labile N-protecting group that still had ortho-directing potential. 3-Aminophenol (**6**) was protected as the *tert*-butyl urethane and then converted to its methoxymethyl ether to yield **7**. However, lithiation[13] of **7** with *tert*-butyllithium at −20 °C in THF followed by quenching of the reaction with methyl chloroformate gave the undesired 1,2,4-trisubstituted ester **8** in 20% yield as the only observable product. In an attempt to change the regioselectivity of lithiation, the less bulky methyl urethane **9** was next prepared from **6**. Lithiation followed by quenching with methyl chloroformate now gave a mixture of the desired isomer **10** (33%), the undesired **11** (6%), and unreacted starting material. These efforts are summarized in Scheme III.

As an alternative for controlling the regioselectivity of lithiation, a less bulky methyl ether protecting group, such as in **12**, was considered. Although in developing a synthesis of **3** from **12** subsequent removal of the methyl ether with Lewis or protic acids had previously been problematic,[10] in a model study it was now found that lithium thiomethoxide[14] in DMF cleanly demethylated **12** in 75% yield to give **13**.

Piv = COt-Bu

Encouraged by this, we prepared *N*-*t*-Boc-3-anisidine (**14**, 98%); lithiation and quenching with CO_2 led to a 3:1 mixture of **15** and **16** (60% combined yield), which unfortunately could be separated only with difficulty. Nevertheless, **15** was treated with concentrated HCl, and the resulting amino acid **17** was treated with phosgene to give the isatoic anhydride **18**, but this provided only a very low yield of the carboxamide **19** even when treated with ammonia in large excess at elevated temperature in a sealed tube. However, when the mixture of **15** and **16** was reacted at −20 °C with methyl chloroformate/triethylamine to give the mixed anhydride[15] and quickly quenched (within 5 min) with anhydrous ammonia, an almost quantitative conversion of **15** to carboxamide **20** was obtained. The undesired isomer **16** apparently reacted much more slowly and was subsequently removed from the crude product by extraction with aqueous bicarbonate.[16] Whereas direct treatment of **20** with lithium thiomethoxide in DMF resulted in cyclization to the imide, stepwise deprotection first with HCl/HOAc and then with the thiomethoxide finally gave the target compound **4** in 70% yield, which was characterized as the crystalline derivative **22**. Scheme IV summarizes these reactions.

[$^{13}CO^{15}NH_2$]-6-Hydroxyanthranilamide was next prepared following the described technology (vida supra). Thus, **14** was lithiated and quenched with $^{13}CO_2$ generated from $BaCO_3$.[17] The mixture of acids was treated with $ClCO_2Me$ in the presence of Et_3N, followed by addition of $^{15}NH_3$ in THF generated from $^{15}NH_4Cl$[18,19] to yield the doubly labeled amide **20a**. Stepwise deprotection gave the doubly labeled amide **4a**.

(9) (a) Gould, S. J.; Chang, C. C.; Darling, D. S.; Roberts, J. D.; Squillacote, M. *J. Am. Chem. Soc.* **1980**, *102*, 1707–1712. (b) Gould, S. J.; Chang, C. C. *Ibid.* **1978**, *100*, 1624–1626. (c) Haber, A.; Johnson, R. D.; Rinehart, K. L., Jr. **1977**, *99*, 3541–3544.

(10) Hillis, L. R.; Gould, S. J. *J. Org. Chem.* **1985**, *50*, 718–719.

(11) Saá, C.; Guitián, E.; Castedo, L.; Suau, R.; Saá, J. M. *J. Org. Chem.* **1986**, *51*, 2781–2784.

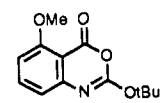
(12) Solvents attempted were THF, EtOH, and CH_2Cl_2, from 40 to 80 °C.

(13) Muchowski, J. M., Venuti, M. C. *J. Org. Chem.* **1980**, *45*, 4798–4801.

(14) Kelly, T. R.; Dali, H. M.; Tsang, W.-G. *Tetrahedron Lett.* **1977**, 3859–3860.

(15) Fukuyama, T.; Li, L.; Laird, A. A.; Frank, R. F. *J. Am. Chem. Soc.* **1987**, *109*, 1587–1589.

(16) If the reaction was carried out at 0 or −78 °C or ammonia added much later than 5 min after the chloroformate, the major product was the cyclized **21**:

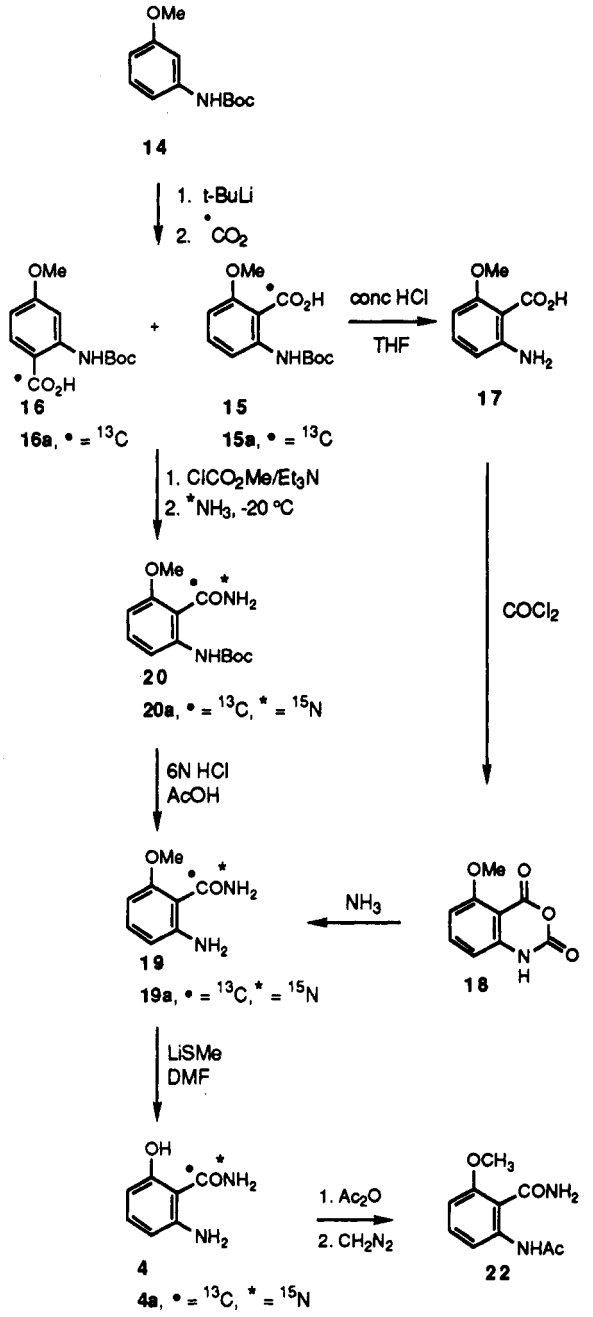


(17) Pomerantz, M.; Fink, R. *J. Labelled Compds. Radiopharm.* **1979**, *16*, 275–286.

(18) Lambert, J. B.; Oliver, W. L.; Roberts, J. D. *J. Am. Chem. Soc.* **1965**, *87*, 5085–5090.

(19) Ott, D. G. *Syntheses With Stable Isotopes of Carbon, Nitrogen, and Oxygen*; John Wiley & Sons: New York, 1981; p 79.

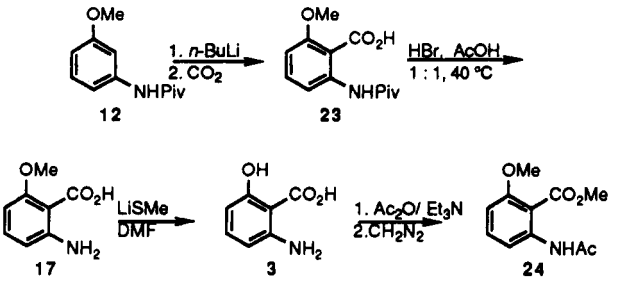
Scheme IV



In the original synthesis of **3**, deprotection of the pivalamide **23** was sometimes erratic, leading to concomitant decarboxylation. Based on the synthesis of **4**, a reliable route to **3** has now been developed from **23**. Hydrolysis with HBr/HOAc at 40 °C for 2 days gave 6-methoxyanthranilic acid (**17**) in 72% yield, and the methyl ether was then cleaved with the thiomethoxide procedure. Chromatography of the crude product on an anion-exchange column gave pure **3** as its acetic acid salt in 56% yield, which was fully characterized as the crystalline derivative **24**. These reactions are summarized in Scheme V.

Biosynthesis. The stage was now set for feeding **4a** to *S. helicus*. We had previously reported[7] that the ^{13}C NMR resonances of different samples of **1** exhibited varying line widths, thus potentially complicating an analysis based on small isotope shifts. We have since found that this is due entirely to concentration effects, as shown in Figure 1 for the concentration dependence of the quinone resonance line widths. At a concentration in DMSO-d_6 of 0.038 M,

Scheme V



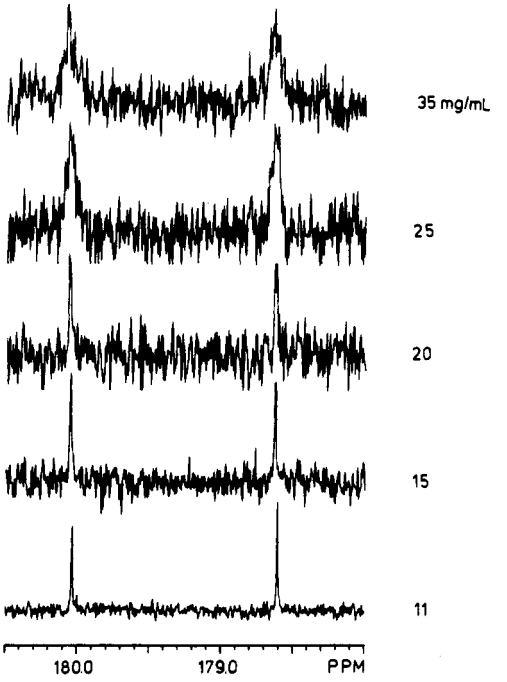


Figure 1. Partial ^{13}C NMR spectra of sarubicin A (**1**) at various concentrations in DMSO-d_6. The resonances shown are due to the quinone carbonyls.

the carbonyl lines at 180.0 and 178.8 ppm were less than 2 Hz wide at the base.[20] At a concentration of 0.085 M, the line widths increased to 15 Hz. This effect was seen to varying extents in all of the ^{13}C resonances except the carboxamide line at 169.5 ppm and the methyl resonance at 16.7 ppm. As the concentration was increased, this trend was followed and the quality of the spectra rapidly degraded.

Labeled **4a** was pulse-fed to growing production cultures of *S. helicus* at 24, 48, and 60 h after inoculation.[7] The fermentation was terminated at 72 h and the antibiotic isolated from the broth. Incorporation of **4a** was immediately evident (Scheme II) from the ^{13}C NMR spectrum of the derived **1b**, which revealed a trio of lines for the carboxamide resonance due to the ^{13}C–^{15}N spin-coupled doublet[1,9] (J = 15.0 Hz) centered 1.5 Hz upfield from the ^{13}C–^{14}N singlet at 169.67 ppm. By comparing line intensities with those of a spectrum of natural abundance **1** (normalizing to C-5 at 154.06 ppm), we determined that the singlet was 1.51 times that expected for just natural abundance, indicating that some hydrolysis of **4a** had occurred. With this correction, a 4.34-fold enrichment from the doublet could be measured. The combined enrichments (4.85-fold) corresponded to a 1.29% incorpo-

(20) Line widths at the base rather than at half-height are relevant in the present case because the level of incorporation of **4a** into **1** could not be accurately predicted, and a low incorporation could have yielded only a small isotope-shifted resonance.

ration of the **4a** fed, of which 10.2% had first been hydrolyzed in vivo to the acid. Therefore, carboxamide formation from the acid **3** is the next step in the biosynthesis of **1**.

Experimental Section

General Procedures. Elemental analyses were performed by Desert Analytical, Tucson, AZ. Tetrahydrofuran (THF) was distilled from potassium benzophenone ketyl just prior to use. Dimethylformamide (DMF) was distilled from CaH_2 and stored over 3-Å sieves. Methylene chloride was distilled from CaH_2 just prior to use. All other solvents were used as obtained. Flash chromatography was carried out on silica gel (EM Reagents, Keiselgel 60, 230–400 mesh) or Silicar CC-4 (Mallinkrodt).

Syntheses. Isatoic Anhydride 5. To a solution of **3** (50 mg, 0.264 mmol) in dioxane (10 mL) were added phosgene (1.93 M in toluene, 0.27 mL, 0.528 mmol) and powdered $NaHCO_3$ (110 mg, 1.32 mmol). The mixture was stirred at 50 °C for 1 h until no starting material was visible by TLC (20% methanol/ethyl acetate). The mixture was filtered and concentrated in vacuo to a white powder, which was carefully washed with cold methanol to yield 44 mg (94%) of **5** as a white powder: mp >300 °C; IR (KBr) 3330–3022, 1765, 1703, 1639, 1516, 1386 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 10.67 (br s, 1 H), 10.12 (br s, 1 H), 7.63 (t, J = 8.0 Hz, 1 H), 6.73 (d, J = 8.0 Hz, 1 H), 6.71 (d, J = 8.0 Hz, 1 H); ^{13}C NMR (acetone-d_6, 100.6 MHz) δ 165.12, 161.91, 146.76, 142.44, 139.39, 111.30, 106.06, 98.32.

***N*-(*tert*-Butoxycarbonyl)-3-[(methoxymethyl)oxy]aniline (7).** To a solution of 3-aminophenol (4.00 g, 36.7 mmol) in THF (200 mL) was added di-*tert*-butyl dicarbonate (9.67 g, 44.3 mmol) in THF (75 mL). The solution was stirred at reflux for 24 h and concentrated in vacuo to give a brown oil. This was dissolved in ethyl acetate and washed with cold 0.5 M HCl, H_2O, and then saturated $NaHCO_3$, followed by saturated brine. The dried solution was concentrated in vacuo to yield a brown oil. After crystallization from CH_2Cl_2/hexane, 7.34 g (96%) of light tan crystals (mp 119.5–120.0 °C) was obtained. A portion of this (3.30 g, 15.8 mmol) was added to a mixture of NaH (0.80 g, 33.2 mmol) in THF (200 mL) at 0 °C. After stirring for 1 h at 0 °C, chloromethyl methyl ether (1.32 mL, 17.3 mmol) was added and the reaction allowed to come to room temperature overnight. The solution was diluted with ethyl acetate and then washed with 5% aqueous NaOH, water, and saturated brine. The dried solution was concentrated in vacuo, and the residue was passed through a flash column (4.5 × 15 cm) eluting with 5% ethyl acetate/CH_2Cl_2 to obtain a light yellow oil (2.56 g, 64%): IR (neat) 3340, 2978, 1730, 1709, 1537, 1393, 1151 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz) δ 8.41 (br s, 1 H), 7.36 (m, 1 H), 7.14 (m, 2 H), 6.65 (m, 1 H), 5.15 (s, 2 H), 3.41 (s, 3 H), 1.46 (s, 9 H); ^{13}C NMR (acetone-d_6, 75.4 MHz) δ 158.73, 153.26, 141.74, 130.14, 112.39, 110.61, 107.22, 94.91, 79.91, 55.92, 28.44; MS (70 eV) m/z 253 (M^+, 97.3), 197 (97.3), 153 (44.8), 121 (48.7), 57 (100), 45 (95.5). Anal. Calcd for $C_{13}H_{19}NO_4$: C, 61.63; H, 7.56; N, 5.53. Found: C, 61.76; H, 7.62; N, 5.43.

***N*-(Methoxycarbonyl)-3-[(methoxymethyl)oxy]aniline (9).** To a solution of 3-aminophenol (5.00 g, 45.9 mmol) in ethyl acetate was added 8% $NaHCO_3$ (150 mL) followed by methyl chloroformate (3.89 mL, 50.5 mmol). The two-phased solution was stirred overnight. After the phases were separated, the organic layer was washed with 1 N HCl and then H_2O, followed by saturated brine, and then dried over $MgSO_4$. Concentration of the organic layer in vacuo yielded a white solid, which was crystallized from CH_2Cl_2/hexanes to yield 7.53 g (94.8%) of colorless crystals: mp 95.0–95.5 °C; IR (KBr) 3407, 3300, 1697, 1541, 1455, 1225 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz) δ 8.57 (br s, 1 H), 8.32 (s, 1 H), 7.21 (t, J = 2.2 Hz, 1 H), 7.09 (t, J = 8.2 Hz, 1 H), 6.97 (ddd, J = 8.1, 2.2, 1.7 Hz, 1 H), 6.51 (ddd, J = 8.2, 2.2, 1.1 Hz, 1 H), 3.68 (s, 3 H); ^{13}C NMR (acetone-d_6, 75.4 MHz) δ 158.66, 154.75, 141.29, 130.28, 110.42, 110.27, 106.21, 51.99; MS (FAB$^+$) m/z 168 (M^+ + 1). Anal. Calcd for $C_8H_9NO_3$: C, 57.47; H, 5.43; N, 8.38. Found: C, 57.33; H, 5.41; N, 8.38.

To a suspension of NaH (1.37 g, 57.0 mmol) in dry THF under an argon atmosphere at 0 °C was added the 3-[*N*-(methoxycarbonyl)amino]phenol in THF. After 1 h at 0 °C, the mixture was quenched with chloromethyl methyl ether (2.25 mL, 29.6 mmol) and stirred at room temperature overnight. The heterogeneous mixture was diluted with ethyl acetate and extracted with 5% NaOH. The organic layer was washed with water and then saturated brine, dried, and then concentrated in vacuo to give a light yellow oil, which was pure by TLC. A small amount was subjected to Kügelrohr distillation to yield a colorless oil: IR (neat) 3326, 2954, 1738, 1716, 1610, 1543, 1151 cm^{-1}; ^1H NMR (acetonitrile-d_3, 400 MHz) δ 7.79 (br s, 1 H), 7.22 (d, J = 1.8 Hz, 1 H), 7.19 (t, J = 8.5 Hz, 1 H), 7.03 (dd, J = 8.5, 1.8 Hz, 1 H), 6.69 (dd, J = 8.5, 1.8 Hz, 1 H), 5.14 (s, 2 H), 3.69 (s, 3 H), 3.41 (s, 3 H); ^{13}C NMR (acetonitrile-d_3, 100.6 MHz) δ 158.74, 155.13, 141.13, 130.66, 112.90, 111.60, 107.58, 95.24, 56.30, 52.66; MS (70 eV) m/z 211 (M^+, 100), 179 (67.9), 45 (97.6). Anal. Calcd for $C_{10}H_{13}NO_4$: C, 56.85; H, 6.21; N, 6.63. Found: C, 56.87; H, 6.37; N, 6.35.

Lithiations. General Procedure for Urethane-Protected Anilines. The protected aniline was dissolved in dry THF under an argon atmosphere and cooled to −78 °C. *t*-BuLi was added and the canary yellow solution stirred for 15 min, then warmed to −20 °C, and stirred for an additional 2–2.5 h, whereupon the appropriate electrophile was added and the reaction mixture allowed to warm slowly to room temperature over 6 h. The workup procedures are described below.

Methyl *N*-(*tert*-Butoxycarbonyl)-4-[(methoxymethyl)oxy]anthranilate (8). As described above, to a stirred solution of **7** (100.0 mg, 0.395 mmol) in dry THF was added *t*-BuLi (1.7 M, 0.79 mL, 1.34 mmol), and the canary yellow solution was stirred and then quenched by the addition of methyl chloroformate (0.30 mL, 0.395 mmol). After dilution with ethyl acetate, the organic layer was washed with water and then saturated brine and then dried. Concentration in vacuo afforded a light yellow oil. Silica gel chromatography eluting with 21% Et_2O/hexanes first yielded **8** (27 mg, 22%) as a colorless oil and then starting material (47 mg, 47% recovered). Physical data for **8**: IR (neat) 3300, 2978, 1732, 1689, 1587, 1264, 1155 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 10.52 (br s, 1 H), 8.18 (d, J = 2.5 Hz, 1 H), 7.96 (d, J = 9.0 Hz, 1 H), 6.73 (dd, J = 9.0, 2.5 Hz, 1 H), 5.28 (s, 2 H), 3.88 (s, 3 H), 3.45 (s, 3 H), 1.53 (s, 9 H); ^{13}C NMR (acetone-d_6, 100.6 MHz) δ 168.96, 163.06, 153.15, 144.97, 133.50, 109.84, 108.68, 105.96, 94.73, 81.02, 56.39, 52.41, 28.37; MS (70 eV) m/z 311 (M^+, 15), 212 (80), 180 (100), 57 (75).

Esters 10 and 11. Methyl urethane **9** (73.5 mg, 0.348 mmol) in THF (3 mL) was treated with *t*-BuLi (1.7 M, 0.70 mL, 1.184 mmol), and the resulting solution was quenched with methyl chloroformate (0.027 mL, 0.348 mmol). The mixture was diluted with ethyl acetate, washed with water, followed by saturated brine, and dried. Concentration in vacuo yielded a yellow oil, which was fractionated with a Chromatotron (1-mm silica gel plate) eluting with 3% ethyl acetate/$CHCl_3$ to yield, in order of elution, the 1,2,4-trisubstituted ester **11** (5.6 mg, 6%) followed by **10** (27.9 mg, 33.0%), each as a clear, colorless oil. Physical data for **10**: IR (neat) 3360, 2955, 1742, 1694, 1599, 1274, 1024 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz) δ 8.64 (br s, 1 H), 7.72 (d, J = 8.4 Hz, 1 H), 7.40, (t, J = 8.4 Hz, 1 H), 6.93 (dd, J = 8.3, 1.1 Hz, 1 H), 5.23 (s, 2 H), 3.89 (s, 3 H), 3.71 (s, 3 H), 3.45 (s, 3 H); ^{13}C NMR (acetone-d_6, 75.4 MHz) δ 168.18, 156.89, 154.56, 139.39, 132.82, 114.62, 111.00, 95.69, 56.36, 52.64, 52.49; MS (70 eV) m/z 269 (50), 193 (100), 176 (30), 161 (71), 135 (18), 107 (15); HRMS calcd for $C_{12}H_{15}NO_6$ 269.089 95, found 269.089 90. Physical data for **11**: ^1H NMR (acetone-d_6, 400 MHz) δ 10.69 (br s, 1 H), 8.17 (d, J = 2.4 Hz, 1 H), 7.98 (d, J = 8.9 Hz, 1 H), 6.77 (dd, J = 8.9, 2.4 Hz, 1 H), 5.29 (s, 2 H), 3.89 (s, 3 H), 3.76 (s, 3 H), 3.45 (s, 3 H); HRMS calcd for $C_{12}H_{15}NO_6$ 269.089 95, found 269.089 90.

***N*-(*tert*-Butoxycarbonyl)-3-anisidine (14).** To a solution of 3-anisidine (5.95 mL, 52.9 mmol) in THF (250 mL) was added di-*tert*-butyl dicarbonate (12.0 g, 55.0 mmol) in THF (30 mL). The solution was stirred for 48 h at 50 °C. The solvent was evaporated, and the oily red residue crystallized from hexane to yield 11.09 g (94%) of white crystals: mp 57.0–58.0 °C; IR (neat) 3340, 2980, 1728, 1706, 1607, 1238 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 8.37 (br s, 1 H), 7.28 (m, 1 H), 7.16 (t, J = 8 Hz, 1 H), 7.08 (dd, J = 8, 2 Hz, 1 H), 6.57 (dd, J = 8, 2 Hz, 1 H), 3.76 (s, 3 H), 1.48 (s, 9 H); ^{13}C NMR (acetone-d_6, 100.6 MHz) δ 161.14, 153.60, 141.82, 130.18, 111.30, 108.44, 104.91, 79.91, 55.34, 28.48; MS (FAB$^+$) m/z 224 (M^+ + 1, 60), 168 (100). Anal. Calcd for $C_{12}H_{17}NO_3$: C, 64.54; H, 7.69; N, 6.29. Found: C, 64.36; H, 7.69; N, 6.29.

Acids 15 and 16. To a stirred solution of **14** (5.00 g, 22.4 mmol) in dry THF (250 mL) was added *t*-BuLi (1.40 M, 40 mL, 54.9 mmol). The canary yellow solution was stirred (vida supra) and then quenched as follows: Carbon dioxide was passed through $CaSO_4$ and bubbled into the solution for 2 h, with stirring at room temperature. After being stirred for an additional 6 h at room temperature, the solution was extracted twice with 5% NaOH. The aqueous layer was acidified with solid citric acid and extracted three times with ethyl acetate. The combined organic extracts were washed with saturated brine, dried, and concentrated to a red-yellow residue (3.62 g, 60%, 3:1 = **15**:**16** by ^1H NMR). The mixture was used without further purification; however, a small amount was purified by silica gel chromatography for characterization of **15** and **16**. Physical data for **15**: IR (KBr) 3377, 2957, 1698, 1593, 1552, 1383 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.38 (br s, 1 H), 8.08 (d, J = 8.0 Hz, 1 H), 7.51 (t, J = 8.0 Hz, 1 H), 6.88 (d, J = 8.0 Hz, 1 H), 4.06 (s, 3 H), 1.51 (s, 9 H); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 170.56, 157.26, 152.27, 137.59, 128.52, 118.37, 110.77, 106.13, 79.03, 55.75, 28.05; MS (FAB$^+$) m/z 268 (M^+ + 1, 4.4), 194 (30.8), 176 (56.7), 161 (100); HRMS calcd for $C_{13}H_{17}NO_5$ 267.110 71, found 267.110 66. Physical data for **16**: ^1H NMR (DMSO-d_6, 400 MHz) δ 10.70 (br s, 1 H), 8.10 (d, J = 2.0 Hz, 1 H), 8.01 (d, J = 8.0 Hz, 1 H), 6.65 (dd, J = 8.0, 2.0 Hz, 1 H), 3.88 (s, 3 H), 1.52 (s, 9 H); HRMS calcd for $C_{13}H_{17}NO_5$ 267.110 71, found 267.110 68.

***N*-(*tert*-Butoxycarbonyl)-6-methoxyanthranilamide (20).** To a stirred mixture of acids **15** and **16** (595 mg, 445 mg of **15**, 1.67 mmol) in CH_2Cl_2 (50 mL) at −20 °C were added Et_3N (0.90 mL, 6.7 mmol) and then, after 10 min, methyl chloroformate (0.70 mL, 8.9 mmol). The cloudy solution was stirred for 5 min, and then NH_3 was bubbled into the solution for 5 min. After being stirred for another 10 min at room temperature, the cloudy mixture was washed with $NaHCO_3/H_2O$ and saturated brine and dried. Concentration at reduced pressure yielded a yellow-brown oil. Chromatography on silica gel (2.5 × 16 cm) eluting with 5% $CH_3OH/CHCl_3$ yielded **20** as a light tan solid (406 mg, 91% from **15**). An analytical sample was obtained by recrystallization from CH_2Cl_2/hexanes: mp 145.0–146.0 °C; IR (KBr) 3475, 2984, 1707, 1600, 1160, 1027 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 11.22 (br s, 1 H), 8.02 (d, J = 8.2 Hz, 1 H), 7.91 (br s, 1 H), 7.37 (t, J = 8.2 Hz, 1 H), 7.11 (br s, 1 H), 6.76 (d, J = 8.2 Hz, 1 H), 3.94 (s, 3 H), 1.48 (s, 9 H); ^{13}C NMR (acetone-d_6, 100.6 MHz) δ 170.19, 159.30, 153.45, 143.49, 132.98, 112.60, 109.18, 105.61, 80.13, 56.52, 28.41; MS (FAB$^+$) m/z 267 (M^+ + 1), 194 (65), 167 (100); HRMS (FAB$^+$) calcd for $C_{13}H_{19}N_2O_4$ 267.134 57, found 267.134 47.

6-Methoxyanthranilamide (19). The amide **20** (300 mg, 1.13 mmol) was stirred in acetic acid (2.0 mL) and 6 N HCl (2.0 mL) at room temperature for 2 h. After the reaction mixture was neutralized carefully with saturated $NaHCO_3$, the mixture was extracted three times with ethyl acetate. The organic layer was washed with saturated brine, dried, and concentrated in vacuo to yield 177 mg (95%) of a light yellow powder. An analytical sample was crystallized from $CHCl_3$/hexanes to yield light yellow crystals: mp 151.5–152.0 °C; IR (KBr) 3465, 1640, 1607, 1400, 1257, 1140 cm^{-1}; ^1H NMR (acetonitrile-d_3, 400 MHz) δ 7.54 (br s, 1 H), 7.08 (t, J = 8.1 Hz, 1 H), 6.35–6.20 (br s, 2 H), 6.32 (d, J = 8.1 Hz, 1 H), 6.24 (d, J = 8.1 Hz, 1 H), 6.07 (br s, 1 H), 3.83 (s, 3 H); ^{13}C NMR (acetonitrile-d_3, 100.6 MHz) δ 171.10, 160.47, 153.38, 133.01, 111.05, 104.62, 99.43, 56.56; MS (70 eV) m/z 166 (98.1), 149 (100), 122 (67.5), 107 (64.4). Anal. Calcd for $C_8H_{10}N_2O_2$: C, 57.82, H, 6.07; N, 16.86. Found: C, 57.52; H, 5.84; N, 16.57.

6-Hydroxyanthranilamide (4). To a solution of methoxyamide **19** (100 mg, 0.60 mmol) in DMF (4 mL) was added LiSMe (97 mg, 1.79 mmol), and the mixture was stirred at 80 °C for 6 h. The DMF was removed under high vacuum, whereupon the oily residue was dissolved in methanol (5 mL) and diluted with ethyl acetate. The solution was washed with saturated NH_4Cl, water, and then saturated brine. After drying and concentrating in vacuo, a light tan powder was obtained. This was chromatographed on Silicar CC-4 (1.5 × 10 cm) eluting with 5% $MeOH/CHCl_3$ to yield 64 mg (70%) of a light tan powder. An unidentified aromatic impurity (10%) was present, as evidenced by a three-proton aromatic system in the ^1H NMR spectrum: δ 7.75 (t, J = 8.0 Hz), 7.10 (d, J = 8.0 Hz), 6.85 (d, J = 8.0 Hz). A small sample (10 mg) was purified by HPLC [Alltech Econosphere C-18 reversed-phase column (4.6 × 250 mm, 5 μm) connected to a precolumn packed with C-18 30–40-μm material] eluting at 1.0 mL/min with 85:15 H_2O/MeOH, and 0.15 M H_3PO_4. Detection was by UV at 254 nm. Elution of **4** occurred at 6.6 min, while the impurity apparently decomposed during the separation. The aqueous eluant containing **4** was neutralized with solid $NaHCO_3$ and then extracted three times with ethyl acetate. The organic layers were washed with saturated brine, dried, and concentrated at reduced pressure to yield **4** as a light brown powder (8 mg): mp 134.0–135.0 °C; IR (KBr) 3324, 1640, 1616, 1577, 1242 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.73 (s, 1 H), 7.57 (br s, 2 H), 6.88 (t, J = 8.0 Hz, 1 H), 6.64 (br s, 2 H), 6.16 (d, J = 8.0 Hz, 1 H), 6.04 (d, J = 8.0 Hz, 1 H); ^{13}C NMR (acetone-d_6, 100.6 MHz) δ 170.35, 157.62, 152.09, 136.20, 131.35, 107.77, 102.54; UV (MeOH) 330 (ϵ 5350), 233 (23 000), 211 nm (19 000); MS (70 eV) m/z 152 (67.8), 135 (100), 107 (46.9), 79 (29.8); HRMS calcd for $C_7H_8N_2O_2$ 152.058 64, found 152.058 20.

6-Methoxyanthranilic Acid (17). The pivalamide **23** (1.50 g, 5.98 mmol) was dissolved in acetic acid (7 mL), followed by 48% HBr (7 mL), and then stirred under N_2 for 3 days at 40 °C. The volatiles were removed at 40 °C under high vacuum, and the product crystallized from $MeOH/Et_2O$ to yield 1.22 g (82.3%) of **17** (as the hydrobromide salt) as light brown crystals: mp 157.0–157.5 °C; IR (KBr) 3200–3000, 1700, 1621, 1550, 1473, 1285, 1223, 811 cm^{-1}; ^1H NMR (methanol-d_4, 300 MHz) δ 7.61 (t, J = 8.5 Hz, 1 H), 7.25 (d, J = 8.5 Hz, 1 H), 7.07 (d, J = 8.5 Hz, 1 H), 3.93 (s, 3 H); ^{13}C NMR (methanol-d_4, 75.4 MHz) δ 167.75, 160.84, 134.70, 133.84, 116.61, 116.44, 113.22, 57.17; HRMS calcd for $C_8H_{10}NO_3$ (M^+ + 1) 168.066 10, found 168.066 59.

6-Hydroxyanthranilic Acid (3). The acid **17** (495 mg, 2.00 mmol) was dissolved in DMF (11 mL), and LiSMe (650 mg, 12.00 mmol) was then added. After heating at 95 °C for 24 h, the DMF was removed in vacuo. The residue was taken up in 5% NaOH (25 mL). This was washed with ethyl acetate and then applied to a column of Dowex 1X4 (200–400 mesh, 1.5 × 8 cm). The column was washed with water (30 mL) and eluted with 1 M NH_4Cl, pH 8.0 (1-mL fractions at 1 mL/min). Starting material (25 mg) was eluted within the first eight fractions. The column was next washed with water (30 mL) and then eluted with 0.5 N HCl. Fractions containing the amino acid were pooled, extracted exhaustively with ethyl acetate, and then dried. Concentration yielded 235 mg (55.3%) of **3**, as the acetic acid salt: mp 144.0–144.5 °C; IR (KBr) 3300, 1680, 1670, 1635, 1555, 1440, 1180 cm^{-1}; ^1H NMR (methanol-d_4, 400 MHz) δ 7.03 (t, J = 8.0 Hz, 1 H), 6.19 (dd, J = 8.0, 1.1 Hz, 1 H), 6.00 (br d, J = 8.1 Hz, 1 H), 1.90 (s, 3 H); ^{13}C NMR (methanol-d_4, 100.6 MHz) δ 172.87, 172.01, 162.63, 150.53, 134.47, 107.09, 103.33, 98.42, 21.04; MS (70 eV) m/z 153 (M^+, 70), 135 (100), 107 (95), 79 (79); HRMS calcd for $C_7H_7NO_3$ 153.042 61, found 153.042 60.

***N*-Acetyl-6-methoxyanthranilamide (24).** 6-Hydroxyanthranilamide (**4**) was dissolved in acetic anhydride (2 mL) and Et_3N (2 mL) and stirred at rt overnight. Volatiles were distilled under vacuum, and the residue was dissolved in methanol (5 mL). Freshly prepared ethereal diazomethane was added until bubbling ceased, and the mixture was stirred for 1 h. Concentration in vacuo yielded a white powder, which was crystallized from water to yield **24** as colorless crystals: mp 174.0–174.5 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ 12.09 (br s, 1 H), 8.32 (d, J = 8.0 Hz, 1 H), 7.85 (br s, 1 H), 7.40 (t, J = 8.0 Hz, 1 H), 6.70 (d, J = 8.0 Hz, 1 H), 6.26 (br s, 1 H), 3.94 (s, 3 H), 2.19 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 170.06, 169.14, 158.19, 142.59, 132.96, 114.35, 107.51, 105.80, 56.25, 25.53; MS (70 eV) m/z 208 (89), 176 (27), 166 (78), 149 (100), 122 (28), 107 (18). Anal. Calcd for $C_{10}H_{12}N_2O_3$: C, 57.67; H, 5.81; N, 13.46. Found: C, 57.59; H, 5.82, N, 13.33.

Labeled Syntheses. [$^{13}CO_2H$]-*N*-(*tert*-Butoxycarbonyl)-6-methoxyanthranilic Acid (15a). $Ba^{13}CO_3$ (99% ^{13}C, 4.44 g, 22.42 mmol) was placed into a flask fitted with a dropping funnel containing concd H_2SO_4, which was in turn fitted with a vacuum adapter and a U-tube filled with $CaSO_4$. This was fitted to another flask charged with the urethane **14** (5.00 g, 22.42 mmol) in anhydrous THF (175 mL). The apparatus was purged with N_2 and the urethane solution cooled to −78 °C. *t*-BuLi (1.40 M, 40 mL, 55 mmol) was added and the solution stirred for 15 min, then warmed to −20 °C, and stirred for 2.5 h. The yellow solution was frozen with liquid N_2 and evacuated to 0.25 mmHg, and the system was then closed to the vacuum pump. Generation of $^{13}CO_2$ was accomplished by careful addition of H_2SO_4 to the

flask containing the $Ba^{13}CO_3$. When CO_2 evolution had ceased, the THF solution was allowed to warm until just thawed, then refrozen, and finally allowed to warm to room temperature over 6 h. Workup of the reaction mixture was carried out as previously described for the unlabeled material to yield 3.27 g (54.7% overall) of a 3:1 mixture of isomers **15a** and **16a** (by ^1H NMR). TLC analysis and ^1H NMR data were identical with those of unlabeled **15** and **16** described above. The mixture was used without any further purification.

[$^{13}CO^{15}NH_2$]-*N*-(*tert*-Butoxycarbonyl)-6-methoxyanthranilamide (20a). To a flask fitted with a reflux condenser was added $^{15}NH_4Cl$ (99% ^{15}N, 415 mg, 7.46 mmol) dissolved in water (1 mL), and the system was then purged with N_2. NaOH (3 g) in water (5 mL) was added and the mixture heated to 110 °C. The $^{15}NH_3$ generated was swept with N_2 for 35 min through NaOH pellets and into an anhydrous THF solution (25 mL) which was cooled to −78 °C.

To a 3:1 mixture of acids **15a** and **16a** (2.00 g, 1.50 g of **15a**, 5.6 mmol) in dry THF (60 mL), cooled to −20 °C, was added Et_3N (3.12 mL, 22.4 mmol) followed by methyl chloroformate (2.31 mL, 29.84 mmol). After stirring for exactly 5 min, the previously prepared $^{15}NH_3$ solution, which had been warmed to −42 °C, was transferred via cannula into the solution containing the activated acids. The resulting white slurry was stirred at −20 °C for 30 min and then at rt for an additional 20 min. The product **20a** was isolated as described for **20** to yield from the silica gel column 952 mg (63.5%) of a light tan powder: mp 145.0–146.0 °C; ^1H NMR and ^{13}C NMR spectra of a sample diluted 1:10 with a natural abundance sample of **20** were identical to those described for **20** except that the now intense carboxamide resonance at δ 170.19 was split into a doublet, J = 15.1 Hz, centered on 170.17 ppm in the latter spectrum; long-range couplings to natural abundance carbons could not be observed with this dilution.

[$^{13}CO^{15}NH_2$]-6-Methoxyanthranilamide (19a). The doubly labeled **20a** (300 mg, 1.12 mmol) was dissolved in glacial acetic acid (2 mL) and 6 N HCl (2 mL) and then stirred at room temperature for 2 h. Workup of the mixture was accomplished as for **19** to give 178 mg (94.7%) of a white powder: mp 151.0–152.0 °C; MS (70 eV) m/z 168 (83), 150 (100), 136 (4), 123 (34), 108 (38). The rest of the physical data were identical with those for **19** except that the carboxamide ^{13}C resonance was a doublet of J = 15.1 Hz centered on 171.08 ppm.

[$^{13}CO^{15}NH_2$]-6-Hydroxyanthranilamide (4a). To a solution of doubly labeled methoxy amide **19a** (160 mg, 0.95 mmol) in DMF (4 mL) was added LiSMe (205 mg, 3.81 mmol), and the mixture was stirred at 80 °C for 6 h. Workup yielded 90 mg (61.5%) of a white powder after silica gel column chromatography as described for **4**. This was used without further purification for the feeding experiment. The physical data (IR, ^1H NMR, ^{13}C NMR) were identical to those for **4** except that the ^{13}C carboxamide resonance was a doublet of J = 15.1 Hz centered at 170.34 ppm. MS (70 eV): m/z 154 (62), 136 (100), 108 (62), 79 (44).

Incorporation of [$^{13}CO^{15}NH_2$]-6-Hydroxyanthranilamide (4a) into Sarubicin A by *S. helicus*. The fermentation conditions have been previously described.[6] The seed medium for the seed culture was prepared from Pharmamedia (2.5 g) and glucose (2.5 g) in tap water (100 mL) in a 500-mL flask. The pH was adjusted to 7.2 with 1 N NaOH and the mixture autoclaved at 121 °C for 20 min. The seed medium was inoculated via sterile loop transfer with spores of *S. helicus* and shaken at 225 rpm and 29 °C for 24 h. Meanwhile, the production medium was prepared by taking up glucose (5.0 g), $(NH_4)_2SO_4$ (1.0 g), $CaCO_3$ (5.0 g), and trace salts (1.0 mL) in deionized water to 1.0 L. The trace salts consisted of $MgSO_4 \cdot 7H_2O$ (200 g), $MnSO_4 \cdot H_2O$ (5.0 g), $ZnSO_4 \cdot H_2O$ (10.0 g), $FeSO_4 \cdot 7H_2O$ (6.0 g), $CoCl_4 \cdot 6H_2O$ (2.0 g), and deionized water to 1 L. Four production flasks (200 mL of broth each in 1-L Erlenmeyer flasks) were each inoculated with 10 mL of seed culture. The doubly labeled **4a** (80.0 mg, 0.467 mmol) was dissolved in water (25 mL) and adjusted to pH 3 to facilitate dissolution. To each of three flasks was added one-third of the solution of **4a** (2.5 mL) at 24 h, followed by identical aliquots at 36 h and 48 h. The fourth flask was a control, and identical volumes of water (pH 3) were added at the appropriate times. The fermentations were stopped at 72 h by the addition of concd HCl. After the flasks that had been fed **4a** were combined, the mixture was filtered, and the filtrate was saturated with $(NH_4)_2SO_4$ (250 g) and extracted with ethyl acetate (250 mL) four times. The combined extracts were washed with saturated brine, dried, filtered, and concentrated in vacuo. The residue was chromatographed on Silicar CC-4 (1.8 × 10 cm) eluting initially with CH_2Cl_2 (50 mL) and then with 1–5% MeOH in CH_2Cl_2 to yield, after concentration, a dark red residue. Crystallization from $CHCl_3$ yielded 33.0 mg (95.5 mmol) of **1b** as bright orange crystals. A 4.5-mg sample of **1b** was analyzed by WALTZ decoupled ^{13}C NMR spectroscopy (DMSO-d_6, 100.6 MHz, SW 25 000 Hz, SI = TD = 64 K, AQ = 1.31 s, PW = 36°, NS = 25 640). An unlabeled sample was run under identical conditions for standardization, (NS = 20 746). The two spectra were identical except for the carboxamide resonance (169.67 ppm) of **1b**, which was flanked by a ^{15}N–^{13}C doublet (J = 15.0 Hz) shifted 1.5 Hz upfield. The ^{13}C enrichment was calculated by normalizing both spectra to C-5 (154.06 ppm).

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Supplementary Material Available: ^1H NMR spectra of **4**, **5**, **8**, **10**, **17**, and **20** (6 pages). Ordering information is given on any current masthead page.