Structure of Amiprilose Hydrochloride, a Novel Anti-inflammatory Agent

R. J. LINHARDT, N. C. BAENZIGER, and B. RONSEN

Received January 23, 1989, from the Division of Medicinal and Natural Products Chemistry, College of Pharmacy and the Department of Chemistry, The University of Iowa, Iowa City, Iowa 52242, and Greenwich Pharmaceuticals, Research Center, 2261 West Campbell Park Drive, Chicago, IL 60612. Accepted for publication June 8, 1989.

Abstract Amiprilose hydrochloride is a carbohydrate-derived, novel anti-inflammatory agent with potential application in the treatment of rheumatoid arthritis. A spectroscopy-based approach was undertaken to assign both the relative and absolute configuration of its five chiral centers. The fully assigned 1H and 13C NMR spectra of amiprilose hydrochloride were used to establish the relative stereochemistry of four of its five chiral centers held rigid in its furanose ring system. Parallel synthesis of the enantiomer of amiprilose hydrochloride from D-glucose was followed by CD spectropolarimetry to establish that no inversion of chiral centers had occurred in the synthesis. The hydrobromide salt of amiprilose and its enantiomer were prepared and, together with amiprilose hydrochloride, were crystallized. X-ray crystallographic analysis resulted in the assignment of the absolute configuration of all five chiral centers.

Amiprilose hydrochloride (4; Therafectin) is a novel anti-inflammatory drug derived synthetically from D-glucose, and is currently in advanced phase II/III clinical trials. Preclinical studies, amiprilose hydrochloride has been shown to affect inflammation and to increase the killing activity of peritoneal-derived lymphocytes. Additionally, the substance suppresses the appearance of Type II collagen-induced joint arthritis in rats. This drug is synthesized from D-glucose (1) by converting it to α-1,2,5,6-di-O-isopropylidene glucofuranose (2), thus leaving only the 3-hydroxyl group unblocked. Following 3-O-alkylation with 1,2,6-N,N-dimethylamino-3-chloropropane (3), the 5,6-O-isopropylidene blocking group is removed (Scheme 1).

Amiprilose hydrochloride is one in a growing list of carbohydrate-based drugs, including polysaccharides, glycocides, nucleosides, and derivatives of simple sugars, containing a high level of chirality. In the past, chemists relied heavily on degradation analysis to characterize such carbohydrates. Alternative approaches used to define chirality, such as X-ray crystallography or the parallel synthesis of an enantiomer of a molecule, are not always possible. Advances in modern NMR spectroscopy have made it possible to unequivocally assign the signals of all the protons and carbons present in a complex molecule such as a carbohydrate. Nuclear magnetic resonance (NMR) spectroscopy can also be used to assign the chiral centers of the rigid chiral centers located within the carbohydrate ring.

It was with the goal of developing such an integrated spectroscopic approach for the analysis of complex carbohydrate drugs that this project was assigned to the relative and absolute stereochemistry of amiprilose hydrochloride was undertaken.

Experimental Section

Materials—Amiprilose hydrochloride (1,2-O-isopropylidene-3-O-3'-{N',N'-dimethylaminomethyl-propyl}-O-D-glucopyranose hydrochloride; 4; lot #17267), its enantiomer (1,2-O-isopropylidene-3-O-3'-{N',N'-dimethylaminomethyl-propyl}-O-D-glucopyranose hydrochloride), 1,2,5,6-di-O-isopropylidene-3-O-3'-{N',N'-dimethylaminomethyl-propyl}-O-D-glucopyranose hydrogen sulfate (3), its enantiomer (1,2,5,6-di-O-isopropylidene-3-O-3'-{N',N'-dimethylaminomethyl-propyl}-α-L-glucopyranose), and 1,2,5,6-di-O-isopropylidene-α-L-glucofuranose were obtained from Greenwich Pharmaceuticals, Chicago, IL. The 1,2,5,6-di-O-isopropylidene-α-D-glucopyranoside (2), D-glucopyranose (1), and L-glucopyranose were from Fluka Chemicals, Milwaukee, WI. All other chemicals and solvents were reagent grade.

Instrumentation—The NMR spectroscopy was performed at 80 MHz proton, 20.15 MHz carbon on an IBM NMR 380 spectrometer and at 360 MHz proton, 90.56 MHz carbon on a Bruker WM360 spectrometer. Low-resolution mass spectrometry was performed on a Fisons Magexpert 20H ion trap mass spectrometer. High-resolution mass spectrometry was performed on a VG ZAB-HF mass spectrometer with VG 11-205f data system and a PEP-11/37 computer. Fourier transform-IR was performed by diffuse reflectance on a Nicolet Fourier transform infrared spectrometer. X-ray crystallography was performed on an Enraf-Nonius CAD-4 diffractometer. Optical rotations were measured using a Perkin-Elmer 141 polarimeter. Circular dichroism (CD) spectropolarimetry was performed on a Jasco model J-500A spectropolarimeter having a 170 to 700 nm wavelength range. Melting points were performed on a Thomas Water capillary melting point apparatus.

Preparation of Derivatives—Amiprilose—Amiprilose hydrochloride (15 g) was dissolved in distilled water (100 mL) and the pH of the solution was adjusted from 6 to 11.6 using 5 M NaOH solution. The resulting solution was extracted three times with 100 mL of chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure affording a colorless, viscous oil. After drying for 24 h under reduced pressure ~12 g of amiprilose was obtained.

Amiprilose Hydrobromide—The acetonate form of anion exchange resin (25 mL) was packed into a column (2 × 10 cm) pretreated with 100 mL of saturated potassium bromide solution and washed with 5 × 100 mL of distilled water. Amiprilose hydrochloride (or its enantiomer), 1 g in 5 mL of water, was then applied to the column. The column was washed with 10 mL of distilled water and the wash was collected, frozen, and freeze-dried, resulting in a clear oil which was solidified by triturating with a mixture of hexane:diethyl ether. After drying under reduced pressure, 1 g of white powder was recovered.

Nuclear Magnetic Resonance (NMR) Analysis—The NMR was performed on the hydrochloride and hydrobromide salts of amiprilose in deuteromethanol at concentrations from 0.1 to 0.5 M using ~1%
tert-amethylisilane internal standard. The NMR spectrum of amiprile was obtained in deuterochloroform at 3.0 M using -1% tert-amethylisilane as an internal standard. The delayed decoupling experiment was performed in amiprilo hydrochloride (0.75 M) in deuteroethanol at 360 MHz. Two-dimensional (2D) decoupled 1H homonuclear correlation spectroscopy (COSY) was performed on amiprilo (2.0 M in deuteroethanol) at 360 MHz. The 2D 1H,13C heteronuclear COSY was performed on amiprilo hydrochloride (0.75 M in deuteroethanol) at 80 MHz 1H, 20.15 MHz 13C. Nuclear Overhauser (NOE) spectroscopy was performed on amiprilo hydrochloride (0.75 M in deuteroethanol) at 360 MHz.

Infrared (IR) Analysis—Fourier transform IR analysis was performed by diffuse reflectance on amiprilo hydrochloride and its enantiomer (200 µg) in KBr (1 mg).

Mass Spectroscopy (MS) Analysis—Low resolution gas chromatography-mass spectrometry (GC-MS) was performed using a 5% phenyl methyl silicone GC column (0.32 mm x 35 m) mounted on a low-resolution mass spectrometer. High-resolution fast atom bombardment (FAB)-MS was performed on amiprilo hydrochloride and its enantiomer in the positive ion mode from a glycerol matrix on a stainless steel target using a standard VB-FAB source with Xe fast atoms at 8 keV with 1 mamp current.

Circular Dichroism (CD) Analysis—The CD spectra of amiprilo hydrochloride (and its enantiomer) and intermediates in their synthetic pathways were obtained in distilled water (pH 7.0) at 25 °C at a concentration of from 30 to 300 g/L.

Optical Rotations—Optical rotations were measured on the Na D-lines in water at concentrations of 25–50 mg/mL using a 1-cm path length quartz cell.

Elemental Analysis—Elemental analysis (C, H, N, O, and Cl or Br) was performed by Gehrlicher Laboratories, Knoxville, TN.

Crystalization of Amiprilo Hydrochloride, Amiprilo Hydrobromide, and Their Enantiomers—The hydrochloride salts could be crystallized from a variety of solvents including ethanol, isopropanol, 1-butanol, nitromethane, and acetonitrile. The best crystals for X-ray analysis were obtained by adding 500 mg of amiprilo hydrochloride (or its enantiomer) to 10 mL of isopropanol at 60 °C. (Amiprilo hydrochloride, mp 177-178 °C; [α]D = -27.8°, Amiprilo hydrobromide, mp 176-178 °C; [α]D = +23.5°.) The resulting saturated solution was filtered hot through glass wool and allowed to crystallize at 25 °C. The hydrobromide salts could be crystallized from methanol, ethanol, isopropanol, nitromethane, and acetonitrile. The best crystals for X-ray analysis were obtained from ethanol by adding 500 mg of amiprilo hydrobromide (or its enantiomer) to 3.5 mL of absolute ethanol at 60 °C. The resulting saturated solution was filtered hot through glass wool and allowed to crystallize at 25 °C.

Crystallographic Methods—The diffraction data were obtained using monochromated Mo Kα radiation, λ = 0.71073 Å; 205 K data collection; scan, 0.6°/0.3 min/θ; background counts, 25% below and above range, peak counting time/background counting time = 2:1, and a scan speed of 1–4 deg/min based on intensity. Cell dimensions were obtained from at least 20 reflections in the 2 (θ) range of 35–40°. The remaining experimental parameters are summarized in Table I. X-ray crystal structures were obtained for amiprilo hydrochloride, amiprilo hydrobromide, and its enantiomer. The structure was initially solved by direct methods from diffraction data from the D-amiprilo hydrobromide. Although all but six hydrogen atoms were located from difference electron density maps, full matrix refinement was carried out with the hydrogen atoms in fixed positions. After least squares refinement, refinement was repeated based on a model with the opposite hand. The agreement indices, summarized in Table I, clearly fix the correct hand of the molecule. The refinement procedures were identical for the L-amiprilo hydrobromide, except that refinement began with starting parameters derived from the D-enantiomer. The data from the D-amiprilo hydrochloride permitted the refinement of the hydrogen atom positions (isotropically) and experimentally verified the calculated hydrogen atom positions assumed in the refinement of the bromide analogue. Again, both D- and L-form models were refined, and the results (in Table I) again indicate the correct choice of models to be the D form, in agreement with the bromide analogue. The small difference in agreement factors for the two enantiomeric forms in the chloride case might not be considered conclusive evidence of the correct handedness by itself. But, since this agrees with the unequivocal results obtained from the bromide analogue, this refinement lends support to the possibility of determining the correct handedness with a rather small amount of additional supporting evidence.

The positional parameters and selected bond distances and angles for D-amiprilo hydrochloride are given in Tables II and III, and the

Table I—Crystallographic Parameters of Amiprilo Salts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D-Amiprilo • HCl</th>
<th>L-Amiprilo • HBr</th>
<th>D-Amiprilo • HBr</th>
</tr>
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<tbody>
<tr>
<td>a</td>
<td>10.74(2)</td>
<td>10.73(2)</td>
<td>10.572(4)</td>
</tr>
<tr>
<td>b</td>
<td>10.769(2)</td>
<td>10.73(2)</td>
<td>10.605(4)</td>
</tr>
<tr>
<td>c</td>
<td>15.869(3)</td>
<td>15.868(2)</td>
<td>15.487(3)</td>
</tr>
<tr>
<td>Vol.</td>
<td>1809.6</td>
<td>1800.1</td>
<td>1736.3</td>
</tr>
<tr>
<td>FW, Z</td>
<td>335.30, 4</td>
<td>335.30, 4</td>
<td>340.84, 4</td>
</tr>
<tr>
<td>d, gm/L</td>
<td>1.144</td>
<td>1.422</td>
<td>1.304</td>
</tr>
</tbody>
</table>

Data Collection
Decay: 10% 22.7 %<2% 1.3%
Lin. Absorp. (1/cm): <2% 22.7 %<2% 1.3%
Absorp. Corr.: None
Method: Temperature
Min-Max: 1.4-2.09
Equlv. Refl.:
Agrae. F. %P: 1.9, 2.1 2.2, 2.7
Total Refl.: 11936 11943 12225
Abs.: 3436 2094 557
After Aver.: 3196 3209 3082
>3σ: 2145 2526 2467

Refinement
Model: H atoms fixed
Parameters: 199 190 312
SDOUW: 1.08 1.11 1.12
α Erant 1, α 2 0.031, 0.061 0.055, 0.089 0.029, 0.025
L Erant 1, α 2 0.005, 0.09 0.020, 0.024 0.022, 0.027
Par. sh/ed.: 0.15 0.16 0.01
Psd. el. dens.(A2): <06 <06 <06
Weighting:
Q = 0.00, P = 0.005
Q = 0.00, P = 0.00
Q = 0.00, P = 0.00

a Space group = P21,21. b For all salts: 29 = 2–60; cells: α = α1; size = 0.1–0.5 x 0.3–0.4 x 0.3–0.6 mm; boundary faces (0,0,0) x 1, 1, 0; refinement = anisotropic, non-H. * See ref 15. † See ref 16.

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Results and Discussion

Amiprilose hydrochloride had a melting point range of 177 to 178 °C and the following composition: C, 49.07%; H, 8.15%; N, 4.03%; O, 28.09%; Cl, 10.11% (theoretical: C, 49.19%; H, 8.26%; N, 4.10%; O, 28.08%; Cl, 10.37%) for C₁₉H₂₅O₆N₆Cl. Low-resolution GC-MS of amiprilose showed a single GC peak with corresponding M + 1 ion in the MS of 306 mass units. High-resolution FAB-MS analysis gave a molecular ion of 306.192 (Δm = 0.1 mmu) corresponding to an M + 1 ion with a molecular formula for the free base of C₁₉H₂₅O₆N₆. The MS analysis is consistent with the calculated molecular mass of 341.834 for amiprilose hydrochloride. The enantiomer of amiprilose hydrochloride had an identical mass spectrum, with an M + 1 ion of 306.189 (Δm = 2.3 mmu).

Proton and carbon NMR of amiprilose hydrochloride in deuteromethanol at 0.75 M at 360 and 90.56 MHz showed signals corresponding to each of the 12 nonequivalent protons and 13 nonequivalent carbons, respectively (Table IV). Delayed decoupling spectrum was used to determine the different types of carbon (i.e., methyl, methylene, methine, and quaternary) corresponding to each signal in the carbon spectrum (Table IV). The anomeric proton H1 was tentatively assigned to the peak at 5.87 ppm based on a reported shift of 5.85 ppm for H1 in the structurally similar 1,2:5:6-di-O-isopropylidene-a-D-glucopyranose (2). Adjacent protons exhibiting connectivity (H1-H3 and H10-H12) in amiprilose (4; the free base was over four times more soluble in organic solvents than the hydrochloride salt) were assigned

<table>
<thead>
<tr>
<th>Atom</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>E(A2)</th>
</tr>
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<tr>
<td>C1</td>
<td>0.5650(4)</td>
<td>0.12153(4)</td>
<td>0.36419(3)</td>
<td>4.155(8)</td>
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<tr>
<td>C1</td>
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<td>0.425(1)</td>
<td>0.7744(6)</td>
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<td>0.38177(1)</td>
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<td>0.2065(1)</td>
<td>0.9144(6)</td>
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<td>C4</td>
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<td>0.1910(1)</td>
<td>0.7019(0)</td>
<td>3.072(1)</td>
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<td>0.3649(2)</td>
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<tr>
<td>C13</td>
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<td>0.8070(1)</td>
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<tr>
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<tr>
<td>C16</td>
<td>0.3104(2)</td>
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<tr>
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<td>0.646(1)</td>
<td>0.7022(0)</td>
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</table>

*All hydrogen atoms were refined isotropically; anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter which is defined as follows: 4a/3 - a²•B(1,1) + b²•B(2,2) + c²•B(3,3) + ab(cos γ)•B(1,2) + ac(cos β)•B(1,3) + bc(cos α)•B(2,3) where a, b, and c are reciprocal lattice constants.

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Table III—Interatomic Distances and Bond Angles for Amiprilose Hydrochloride

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Bond Distance, Å</th>
<th>Atoms</th>
<th>Bond Angle, °</th>
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<tr>
<td>C1-C1</td>
<td>1.401(2)</td>
<td>C1-C1-C2</td>
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<td>C1-C2-C2</td>
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<tr>
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<td>C2-C1-C2</td>
<td>103.8(1)</td>
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<tr>
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<td>1.520(2)</td>
<td>C1-C2-C3</td>
<td>103.8(1)</td>
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<tr>
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<td>C2-C3-C4</td>
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<td>C1-C2-C7</td>
<td>109.9(1)</td>
</tr>
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<td>C11-C1</td>
<td>1.514(2)</td>
<td>C2-C1-C2</td>
<td>109.9(1)</td>
</tr>
<tr>
<td>C12-C12</td>
<td>1.503(2)</td>
<td>C2-C3-C7</td>
<td>107.8(1)</td>
</tr>
<tr>
<td>N1-C1</td>
<td>1.504(2)</td>
<td>C3-C4-C10</td>
<td>113.6(1)</td>
</tr>
<tr>
<td>N1-C13</td>
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<td>C3-C4-C10</td>
<td>113.6(1)</td>
</tr>
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<td>110.7(2)</td>
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<td>110.7(2)</td>
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<td>C3-C7-C9</td>
<td>110.1(1)</td>
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<td>C4-C10-C11</td>
<td>112.6(2)</td>
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<td></td>
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<td>C13-N1-C14</td>
<td>111.7(1)</td>
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Figure 1—X-ray structure of amiprilose hydrochloride.

using 2D 1H decoupled homonuclear COSY at 360 MHz. Nuclear Overhauser effect (NOE) spectroscopy (360 MHz) with irradiation at 1.3 and 1.46 ppm and observation of the H-1, H-2, and H-4 signals permitted the assignment of the isopropylidene methyl groups. Proton signals were correlated to their respective carbon signals using 2D 13C,1H heteronuclear COSY. The only signals in the carbon spectrum that could not be correlated were those at 70.22, 68.41, and 112.61 ppm. Delayed decoupling spectroscopy, however, had already given the carbon type for these three signals as methine, methylene, and quaternary, respectively, permitting their assignment to C-5, C-6, and C-7, and thus resulting in the complete, unequivocal assignment of the carbon spectrum. The overlap of the carbon spectrum of amiprilose hydrochloride closely parallels the published shifts and assignments for its precursor 1,2,5,6-di-O-isopropylidene-α-D-glucuronic (2) and thus the complete, unequivocal assignment of the carbon spectrum of amiprilose hydrochloride was achieved.

The complete assignment of carbon and proton signals in the spectra of amiprilose hydrochloride permitted the relative configuration of the chiral centers at C1, C2, C3, and C4 in the rigid glucuronic ring to be established. The coupling constant, J(1,2), of 3.77 Hz demonstrates a syn relationship between H1 and H2. The J(2,3) coupling constant of 0.5 Hz demonstrates an anti relationship between H2 and H3. The coupling constants J(1,2) of 3.8 Hz and J(2,3) of 0.6 Hz are reported for 1,2,5,6-di-O-isopropylidene-α-D-glucarate (2) and showed a similar syn (1,2) anti (2,3) relationship. The J(3,4) coupling constant of 3.15 Hz demonstrates a syn relationship between H3 and H4. Thus, the assignment of the absolute configuration at any of these four centers would result in the assignment of all four centers.

The carbon and proton spectra of 1-glucopyranose (1) and its enantiomer and 1,2,5,6-di-O-isopropylidene-α-D-glucarate (2) and its enantiomer were in each case identical and in each case in agreement with both their structure and the literature. The 1,2,5,6-di-O-isopropylidene-3,0,3'-, and-3'-di-[N,N-dimethylamino-n-propyl]-α-D-glucarate (3) hydrogen sulfate gave a molecular ion at 346.221 (Δm = 2.3 mmu) corresponding to an M+1 ion with a molecular formula for the free base of C27H35O11N. Proton and carbon NMR at 360 and 90.56 MHz gave spectra consistent with the assigned structure (Table IV legend).

To complete this integrated spectral approach to assign the chirality of amiprilose hydrochloride two tasks still remained. The relative configuration of the flexible chiral center of C-5 had to be assigned and the absolute chirality of at least one of the chiral centers in the molecule needed to be established. Although NMR spectroscopy using chiral shift reagents might permit the assignment of the relative chirality of C-5 by analogy to a known compound, such as 1,2-isopropylidene glucuronic, it would do nothing to resolve the larger question of absolute chirality.

The parallel chemical synthesis of amiprilose hydrochloride and its enantiomer from D-glucose and L-glucose was under-
Table IV—Nuclear Magnetic Resonance Spectral Assignments for Amiprilose Hydrochloride (0.75 M in CD₂OD)

<table>
<thead>
<tr>
<th>Atom</th>
<th>Chemical Shift, ppm</th>
<th>Carbon Type</th>
<th>Atom</th>
<th>Chemical Shift, ppm*</th>
<th>Multiplicity</th>
<th>Coupling Constant, Hz</th>
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<tr>
<td>C1</td>
<td>106.27</td>
<td>Methine</td>
<td>H1</td>
<td>5.87</td>
<td>Doublet</td>
<td>J1(2;3) 3.77</td>
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<tr>
<td>C2</td>
<td>82.80</td>
<td>Methine</td>
<td>H2</td>
<td>4.70</td>
<td>Doublet</td>
<td>J2(3;4) 3.57</td>
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<tr>
<td>C3</td>
<td>83.88</td>
<td>Methine</td>
<td>H3</td>
<td>3.95</td>
<td>Doublet</td>
<td>J3(4) 7.95</td>
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<tr>
<td>C4</td>
<td>80.81</td>
<td>Methine</td>
<td>H4</td>
<td>4.08</td>
<td>Doublet of doublets</td>
<td>J4(5) 6.20</td>
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<tr>
<td>C5</td>
<td>70.22</td>
<td>Methine</td>
<td>H5</td>
<td>3.87</td>
<td>Doublet of doublets</td>
<td>J5(6a,5) 5.52</td>
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<tr>
<td>C6</td>
<td>64.87</td>
<td>Methylene</td>
<td>H6a</td>
<td>3.77</td>
<td>Doublet of doublets</td>
<td>J(6a,5) 3.00</td>
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<tr>
<td>C7</td>
<td>112.61</td>
<td>Quaternary</td>
<td>H7</td>
<td>3.62</td>
<td>Singlet</td>
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<tr>
<td>C8</td>
<td>27.04</td>
<td>Methyl</td>
<td>H8</td>
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<td>Singlet</td>
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<tr>
<td>C9</td>
<td>26.47</td>
<td>Methylethyl</td>
<td>H9</td>
<td>1.45</td>
<td>Singlet</td>
<td>J9(10) 11.52</td>
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<tr>
<td>C10</td>
<td>26.47</td>
<td>Methylenec</td>
<td>H10a</td>
<td>3.87</td>
<td>Multiply</td>
<td>J10(11a) 6.25</td>
</tr>
<tr>
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<td>25.66</td>
<td>Methylene</td>
<td>H11</td>
<td>3.62</td>
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<td>J11(12a) 5.52</td>
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<tr>
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<tr>
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<td>H14</td>
<td>2.90</td>
<td>Singlet</td>
<td>J14(15a) 5.52</td>
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* Deuteromethanol showed carbon signals from 46.27 to 49.66 ppm. The tentative assignments for 1,2,5,6-di-O-isopropylidene-3-O-3"-N,N-dimethylamino-3-propyl)-8-glucofranosne hydrogen sulfate carbon spectra are as follows: C1; 106.22; C2; 33.73; C3; 83.16; C4; 82.03; C5; 82.03; C6; 73.56; C7; 112.48; C8; 28.45; C9; 27.08; C10; 67.78; C11; 25.64; C12; 55.20; C13; 43.35; C14; 43.35; C15; 109.75; C16; 25.64; C17; 27.04; and for the proton spectra are as follows: H1; 6.75 (d, J(1,2) = 3.88); H2; 7.40 (d, J(2,3) = 0); H3; 3.69 (m); H4; 4.07 (m); H5; 3.00 (m); H6a, 4.07 (m); H6b, 3.93 (m); H8; 1.44 (s); H9; 1.34 (s); H10a, 3.81 (m); H10b, 3.63 (m); H11; 2.05 (m); H12, 2.25 (m); H13, 2.90 (s); H14, 2.90 (s); H16, 1.39 (s); H17, 1.31 (s).

taken to examine if inversion of configuration took place during the chemical synthesis of amiprilose hydrochloride. The enantiomer of amiprilose hydrochloride had identical FT-NMR, proton and carbon NMR, and FAB-MS spectral properties. Elemental analysis gave the following composition: C, 49.11; H, 8.25; N, 3.87; O, 28.29; and Cl, 10.62 (theoretical, C, 49.19; H, 8.26; N, 4.10; O, 28.08; and Cl, 10.37 for C₂₂H₃₅NO₂Cl). D-Glucopyranose and its enantiomer, L-glucopyranose, show characteristic, opposite, but identical CD curves, as previously reported. The D-glucopyranose derivatives all show the expected negative CD curves at wavelengths of about 200 nm (in contrast to the positive CD curve for D-glucofranosone), while the L-glucopyranose derivatives show identical but positive curves suggesting that no inversion of configuration occurs during the synthesis of amiprilose hydrochloride.

Amiprilose hydrobromide had spectral properties similar to amiprilose hydrochloride. It had a map of 146 to 148°C, [α]D = 22.5°, and the following elemental analysis: C, 43.78; H, 7.18; N, 3.60; O, 24.78; and Br, 29.80 (theoretical, C, 43.53; H, 7.51; N, 3.63; O, 24.85; and Br, 29.69 for C₂₂H₃₅NO₂Br).

The crystal structure determination of the three salts unequivocally establishes the absolute configuration of the molecule. The table of bond distances and angles shows that all the bond distances and angles fall within the usual ranges for these values.

Conclusions

In conclusion, NMR spectroscopy was used to successfully assign the relative configuration of four of the five chiral centers in the carbohydrate-based drug amiprilose hydrochloride. Application of CD spectropolarimetry permitted the absolute assignment of all five chiral centers based on the pure D- and L-glucopyranose starting materials. Finally, X-ray crystallography of the hydrobromide salt of amiprilose and its enantiomer resulted in unequivocal assignment of the absolute configuration of amiprilose.

References and Notes

Acknowledgments
The authors thank Greenwich Pharmaceuticals for their support of this work and Dr. Merchant and Loguathan for their technical assistance.