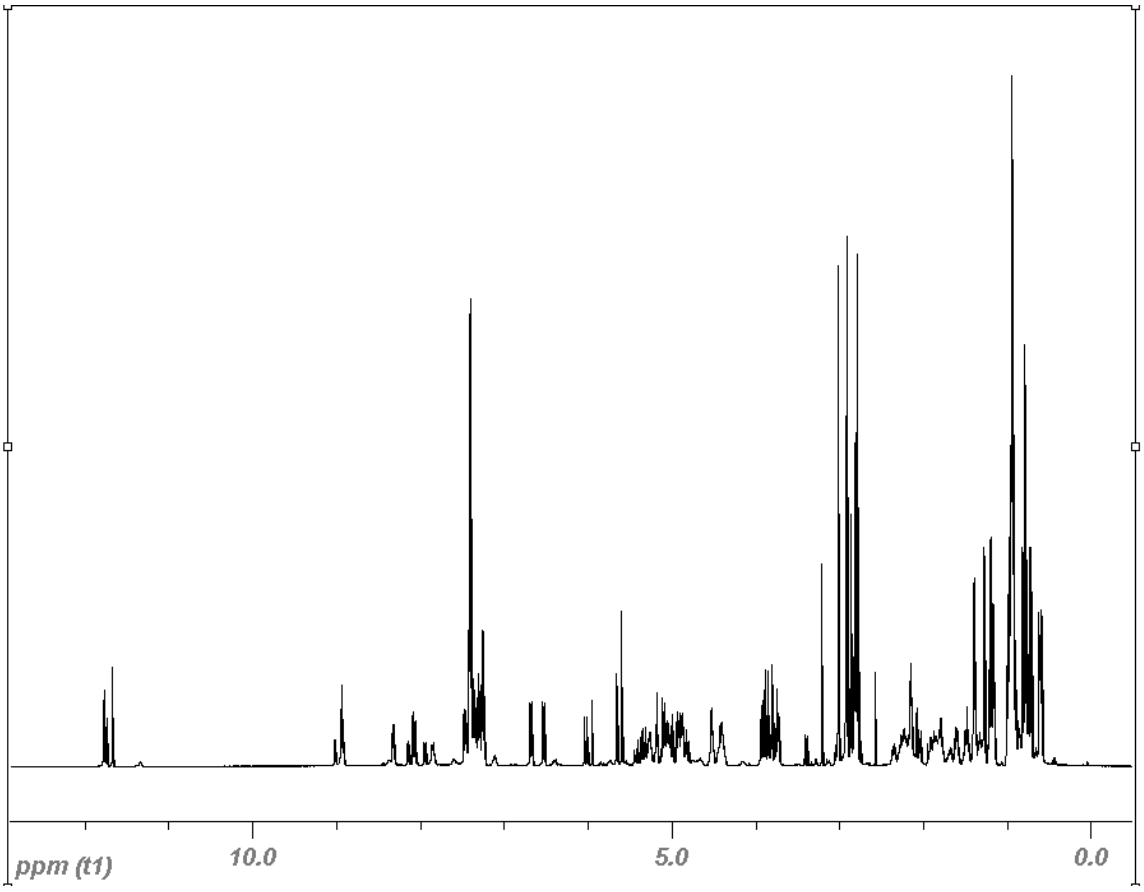
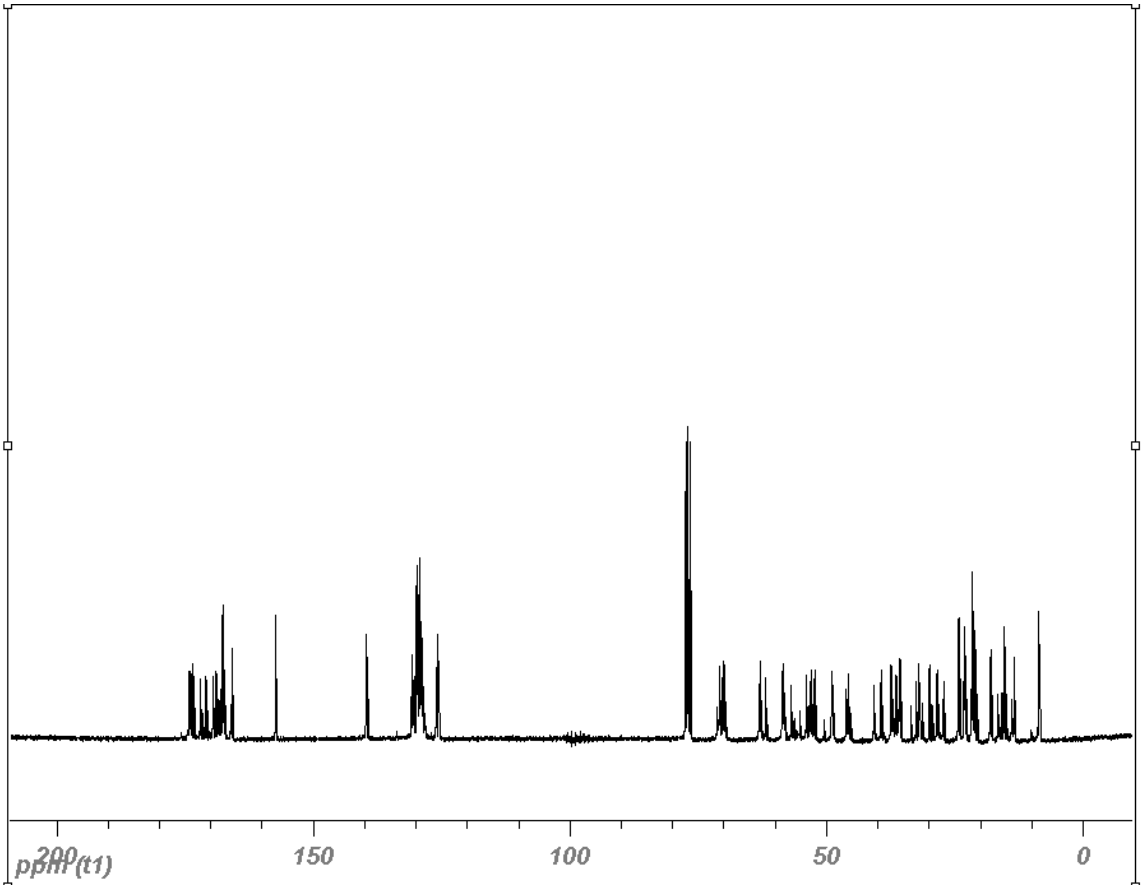
Etamycin: amorphous white powder; $[\alpha]_D^{22}$ +5.8 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (ε) 203 (35 400), 301 (7700) nm; IR (NaCl) ν_{max} 3306, 2956, 1753, 1637 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (125 MHz, $CDCl_3$), and ^{15}N NMR (60 MHz, $CDCl_3$) data, see Table 1; HRESIMS *m/z* $[M + H]^+$ 879.4596 (calcd for $C_{44}H_{63}N_8O_{11}$ 879.4616).

^1H NMR spectrum of etamycin (500 MHz, $CDCl_3$).



^{13}C NMR spectrum of etamycin (125 MHz, $CDCl_3$).



Marfey's Analysis of Etamycin

The absolute stereochemical configuration of etamycin was assigned by acid hydrolysis and application of advanced Marfey's method. Amino acid standards of known configuration were used to confirm the assignment, with the exception of *N*-Me-β-MeLeu (for which no standard was available).

The absolute configuration of **Phg** was determined as *S* (L-Phg). The L-FDLA derivative of Phg in hydrolyzed etamycin was detected by LC/MS analysis at 46.3 min, while the L- and D-FDLA derivatives of Phg in hydrolyzed etamycin were detected by LC/MS analysis at 46.3 and 48.1 min, respectively. Standards of D-Phg and of racemic DL-Phg were also derivatized with L-FDLA. The L-FDLA derivative of D-Phg eluted at 48.3 min, while the L-FDLA derivatives of DL-Phg eluted at 46.4 and 48.3 min.

The absolute configuration of **Ala** was determined as *S* (L-Ala). The L-FDLA derivative of Ala in hydrolyzed etamycin was detected by LC/MS analysis at 37.8 min, while the L- and D-FDLA derivatives of Ala in hydrolyzed etamycin were detected by LC/MS analysis at 37.8 and 41.7 min, respectively. Standards of D-Ala and L-Ala were also derivatized with L-FDLA. The L-FDLA derivative of D-Ala eluted at 41.6 min, while the L-FDLA derivative of L-Ala eluted at 37.6 min.

The absolute configuration of ***N*-Me-β-MeLeu** was determined as *S* (L- *N*-Me-β-MeLeu). The L-FDLA derivative of *N*-Me-β-MeLeu in hydrolyzed etamycin was detected by LC/MS analysis at 50.6 min, while the L- and D-FDLA derivatives of *N*-Me-β-MeLeu in hydrolyzed etamycin were detected by LC/MS analysis at 50.6 and 54.6 min, respectively. No standards were available of *N*-Me-β-MeLeu. However, the elution order was assumed to be the same as observed for Leu, below.

The absolute configuration of **Hyp** was determined as *R* at the α-carbon (D-Hyp), and *S* at the carbon bearing the hydroxyl group (*cis*-D-Hyp). The L-FDLA derivative of Hyp in hydrolyzed etamycin was detected by LC/MS analysis at 31.4 min, while the L- and D-FDLA derivatives of Hyp in hydrolyzed etamycin were detected by LC/MS analysis at 31.1 and 31.4 min, respectively. Standards of *cis*-D-Hyp and *cis*-L-Hyp were also derivatized with L-FDLA. The L-FDLA derivative of *cis*-D-Hyp eluted at 31.3 min, while the L-FDLA derivative of *cis*-L-Hyp eluted at 31.1 min. Because the peaks were so close together, L-FDLA derivatives of each standard were also coinjected with the L-FDLA derivative of hydrolyzed etamycin. The coinjection of L-FDLA-derivatized etamycin with the L-FDLA derivative of *cis*-D-Hyp appeared as a single peak at 31.3 min. The coinjection of L-FDLA-derivatized etamicin with the L-FDLA derivative of *cis*-L-Hyp appeared as two visible peaks, at 31.1 min and 31.3 min. The relative stereochemical configuration at the carbon bearing the hydroxyl group was supported by both (a) the coelution of the L-FDLA derivative with that for a standard of *cis*-D-Hyp (as described above), and (b) a correlation in the NOESY spectrum between H-22 (δ 5.17), and H-24 (δ 4.52), which suggested a *cis* relationship between these hydrogens.

The absolute configuration of **Leu** was determined as *R* (D-Leu). The L-FDLA derivative of Leu in hydrolyzed etamycin was detected by LC/MS analysis at 52.4 min, while the L- and D-FDLA derivatives of Leu in hydrolyzed etamycin were detected by LC/MS analysis at 45.2 and 52.4 min, respectively. Standards of D-Leu and L-Leu were also derivatized with L-FDLA. The L-FDLA derivative of D-Leu eluted at 52.4 min, while the L-FDLA derivative of L-Leu eluted at 45.2 min.

The absolute configuration of **Thr** was determined as S (L-Thr). The L-FDLA derivative of Thr in hydrolyzed etamycin was detected by LC/MS analysis at 32.3 min, while the L- and D-FDLA derivatives of Thr in hydrolyzed etamycin were detected by LC/MS analysis at 32.3 and 37.5 min, respectively. Standards of D-Thr, L-Thr, and DL-allo-Thr were also derivatized with L-FDLA. The L-FDLA derivative of D-Thr eluted at 32.1 min, the L-FDLA derivative of L-Thr eluted at 37.4 min, and the L-FDLA derivatives of DL-allo-Thr eluted at 33.3 and 35.2 min.