Stereoselective Syntheses of the Antibiotics (+)-Negamycin and (−)-HON (RI-331)[1,2]

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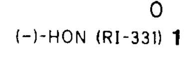
Received 24 June 1991

Publication delayed at the request of the author

The stereoselective syntheses of the antibiotics (−)-HON (5-hydroxy-4-oxo-L-norvaline, RI-331) and (+)-negamycin starting from (*S*)-4-[(*Z*)-2-(benzyloxycarbonylamino)-2-(*tert*-butoxycarbonyl)vinyl]-2,2-dimethyl-1,3-dioxolane and (*R*)-3-benzyloxycarbonyl-5-[(*Z*)-2-(*tert*-butoxycarbonylamino)-2-(methoxycarbonyl) vinyl]-2,2-dimethyl-1,3-oxazolidine, respectively, are described.

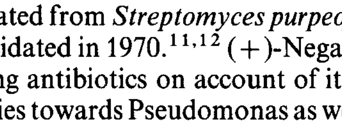
We have recently reported a stereoselective synthesis of (4*R*,2*S*)-4-hydroxyornithine derivatives from (*S*)-2,3-*O*-glyceraldehyde or (*S*)-malic acid, respectively, which included a stereoselective hydrogenation step.[2] The intermediates of this synthesis and their diastereomers can also be employed for the stereoselective preparation of the antibiotics (−)-HON (5-hydroxy-4-oxo-L-norvaline, alternatively known as RI-331) (**1**) and (+)-negamycin (**2**).



(−)-HON (RI-331) **1**

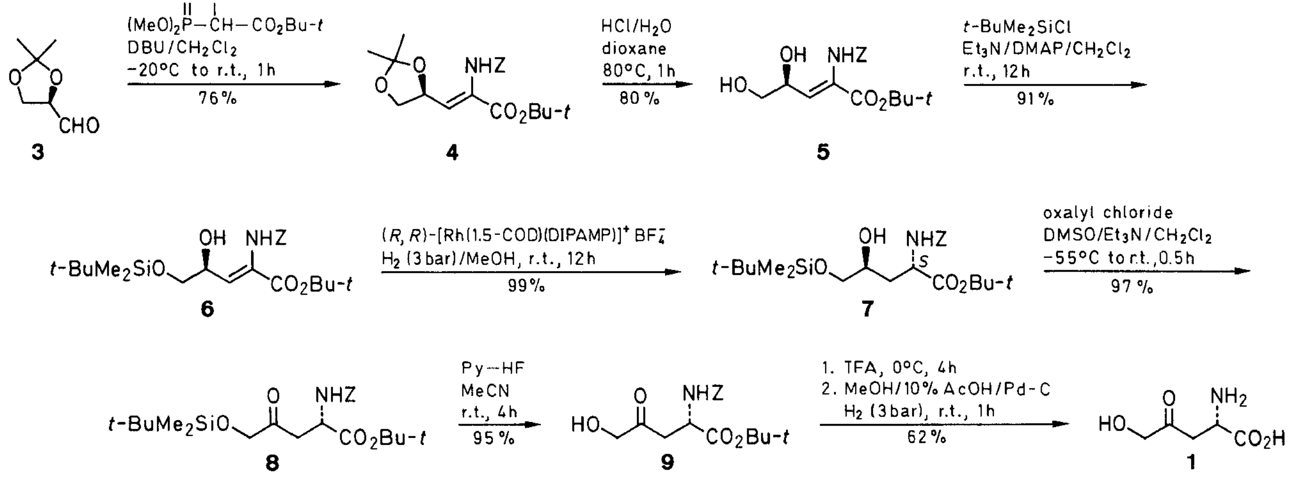
The antibiotic (−)-HON (**1**) was first isolated from *Streptomyces* H-8998 in 1961 and its antibiotic activity towards human- and bovine-types of tuberculum was described.[3,4] It has also been extracted from the culture filtrate of *Streptomyces akiyoshiensis* nov. sp. (under the name RI-331) and its antifungal activity by inhibition of protein synthesis was shown to be the result of a depletion of several amino acids such as threonine, methionine, isoleucine, and serine in the cellular pool.[5-7]

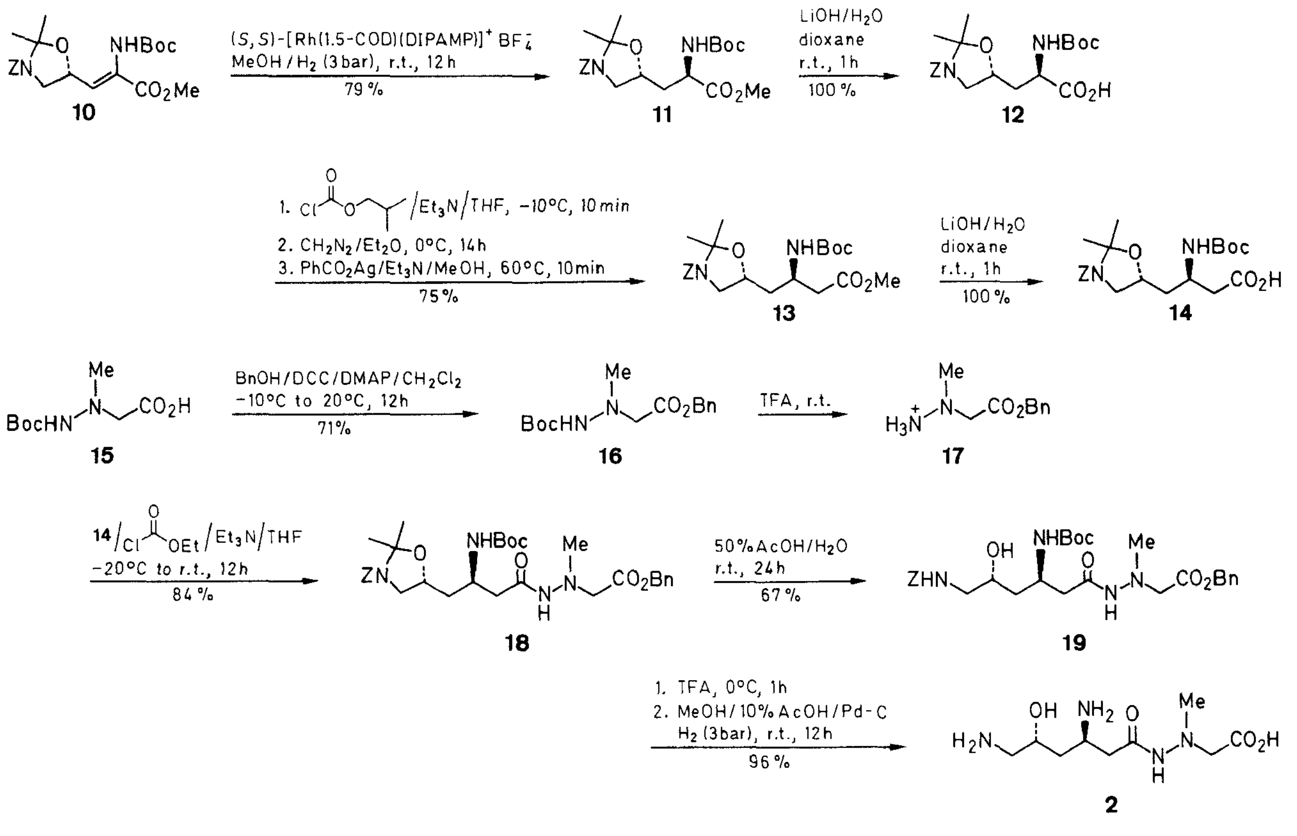
Up to now, however, only the synthesis of racemic HON and the separation of (−)-HON by way of diastereomeric salts has been reported.[8] In the present paper, we describe the stereoselective synthesis of (−)-HON [(−)-RI-331]. *tert*-Butyl (*S*,*Z*)-2-benzyloxycarbonylamino-4,5-isopropylidenedioxy-2-pentenoate (**4**) was obtained by the condensation of (*R*)-2,3-*O*-isopropylideneglyceraldehyde (**3**)[9] with *tert*-butyl 2-benzyloxycarbonylamino-2-(dimethoxyphosphoryl)acetate.[10] The dioxolane derivative **4** was then cleaved to furnish **5** and the primary hydroxy group protected to yield **6**. Subsequent diastereoselective hydrogenation using (*R*,*R*)-[Rh(1,5-COD)(DIPAMP)]$^+$ BF_4^- gave rise to *tert*-butyl (2*S*,4*S*)-2-benzyloxycarbonylamino-5-(*tert*-butyldimethylsiloxy)-4-hydroxypentanoate (**7**). The hydrogenation was completely diastereoselective since the (2*R*,4*S*)-diastereomer could not be detected by HPLC or ^{13}C NMR spectroscopy. Oxidation with oxalyl chloride/dimethyl sulfoxide then generated the completely masked (−)-HON, **8**, which was deprotected by the usual methods. This synthetic (−)-HON (**1**) was identical with the natural compound in all respects.



(+)-Negamycin **2**

(+)-Negamycin (**2**), a rare and unusual peptide antibiotic in that it contains an α-hydrazino acid and a β-amino acid, was isolated from *Streptomyces purpeofuscus* and its structure elucidated in 1970.[11,12] (+)-Negamycin is remarkable among antibiotics on account of its low toxicity and its activities towards Pseudomonas as well as multiple drug-resistant Gram-negative bacteria. It has also been demonstrated to inhibit protein synthesis.[13-16] The first synthesis of (+)-negamycin was reported in 1972.[17] But, just this high activity in combination with a low toxicity





has stimulated considerable recent interest in the stereoselective synthesis of this antibiotic in its optically active form as well as of its diastereomers.[18–23]

Key steps in our synthesis of **2** are a homogeneous diastereoselective hydrogenation and the transformation of an α-amino acid into a β-amino acid via the Wolff rearrangement (Arndt–Eistert transformation). This methodology has been employed for the preparation of β-tyrosine[24] from hydroxyphenylglycine and constitutes the procedure of choice for the synthesis of complicated, acid-sensitive β-amino acids in those cases where the corresponding α-amino acids are accessible.

The didehydroamino acid **10**[2] was hydrogenated using (*S*,*S*)-[Rh(1,5-COD)(DIPAMP)]$^+$ BF$_4^-$ as the catalyst[25,26] to furnish the (*R*)-amino acid derivative **11**. In the hydrogenation reactions of **10** with the rhodium (DIPAMP) catalyst, a significant effect of the chiral center at the 5 position was apparent giving rise to unexpected results. When the (*R*,*R*)-catalyst was employed, 80% of the (5*R*,2*S*)- and 20% of the (5*R*,2*R*)-compounds were formed whereas, with the (*S*,*S*)-catalyst 80% of the (5*R*,2*R*)- and 20% of the (5*R*,2*S*)-compounds were obtained. The ratios of the diastereomers formed were determined by analytical HPLC and the compounds could be purified without difficulty by preparative MPLC. We previously described the hydrogenation of **10**,[2] prepared from (*R*)-2,3-*O*-isopropylideneglyceraldehyde, to furnish the amino acid with a diastereomeric excess of > 99.5%; this result was obtained because the diastereomer was removed by filtration of the crude product through silica gel before the HPLC analysis.

Saponification of the (*R*,*R*)-amino acid derivative **11** and reaction of the mixed anhydride with daizomethane gave rise to the corresponding diazoketone which rearranged smoothly into the β-amino acid with complete conservation of configuration.

Benzyl (1-methylhydrazino)acetate was readily prepared by the reaction of chloroacetic acid with methylhydrazine;[7] isolation of the (1-methylhydrazino)acetic acid as its *tert*-butoxycarbonyl (Boc)-protected derivative **15** was found to be advantageous in this case. The benzyl ester **16** was obtained by dicyclohexylcarbodiimide (DCC) mediated coupling and the Boc group was removed by treatment with trifluoroacetic acid. Transformation to the hydrazide **18** by the mixed anhydride method[28] proceeded in 84% yield. Deprotection of **18** was best achieved by treatment with 50% acetic acid to remove the acetonide group followed by trifluoroacetic acid and catalytic hydrogenation (3 atm of H_2 Pd-C) to furnish crude **2** which was purified over an ion-exchange resin. The spectral properties (^{1}H NMR), TLC behavior, and optical rotation value of the product were identical with the data given in the literature.[11]

The ^{1}H NMR spectra were recorded on Bruker WP 80 (80 MHz) and Bruker AC (250 MHz) spectrometers. Optical rotation values were obtained on a Perkin-Elmer 241 polarimeter. Melting points (Reichert microscope) are not corrected. TLC was performed on silica gel (Merck Silica 650 F_{254} sheets) and medium pressure column chromatography (MPLC) on Merck LiCHroprep Si 60 (15–25 μ). HPLC was carried out using an LKB instrument and a silica gel column (Merck Hibar, LiChrosorb Si 60, 5 μ).

tert-Butyl (*S*,*Z*)-2-Benzyloxycarbonylamino-4,5-isopropylidenedioxy-2-pentanoate (4):

To a solution of *tert*-butyl 2-benzyloxycarbonylamino-2-(dimethoxyphosphoryl)acetate (6.72 g, 18 mmol) in CH_2Cl_2 (30 mL) is added DBU (2.51 g, 16.5 mmol) at r.t. After 15 min the mixture is cooled to −20 °C and **3** (1.95 g, 15 mmol) is added. The mixture is kept for 15 min at − 20 °C and warmed up to r.t. over a period of 1 h. After the solvent is evaporated *in vacuo* the residue is dissolved in EtOAc (50 mL) washed with 1 N aq $KHSO_4$ (30 mL) and sat. aq NaCl (20 mL), dried ($MgSO_4$) and evaporated *in vacuo*. Chromatography on silica gel with petroleum ether (bp 40−60 °C)/EtOAc (7:3) gives the *E*/*Z* mixture (7:93) of **4** as a colorless oil; yield: 4.25 g (76%); $[\alpha]_D^{20}$ −2.1° (*c* = 1.75, $CHCl_3$).

$C_{20}H_{27}NO_6$	calc.	C 63.64	H 7.21	N 3.71
(377.4)	found	63.49	7.41	3.42

^1H NMR (250 MHz, $CDCl_3$/TMS): δ = 1.37 (s, 3 H), 1.47 (s, 3 H), 1.49 (s, 9 H), 3.75−3.90 (m, 1 H), 4.25−4.40 (m, 1 H), 4.75−4.90 (m, 1 H), 5.13 (s, 2 H), 6.35 (d, 1 H, *J* = 8.4), 6.66 (br s, 1 H), 7.36 (s, 5 H).

tert-Butyl (*S*,*Z*)-2-Benzyloxycarbonylamino-4,5-dihydroxy-2-pentenoate (5):

A solution of **4** (7.55 g, 20 mmol) in dioxane (50 mL) and H_2O (50 mL) is treated with 1 N aq HCl (4 mL) and heated for 1 h at 80 °C. Then the dioxane is distilled off and the water layer is extracted with EtOAc (3 × 70 mL). The combined organic layers are dried ($MgSO_4$) and concentrated at reduced pressure to a colorless oil which is purified by chromatography on silica gel using petroleum ether (bp 40−60 °C)/EtOAc (7:3) as eluent; yield: 5.4 g (80%); $[\alpha]_D^{20}$ +10.3° (*c* = 1.11, $CHCl_3$).

$C_{17}H_{23}NO_6$	calc.	C 60.52	H 6.87	N 4.15
(337.4)	found	60.44	6.63	4.19

^1H NMR (80 MHz, $CDCl_3$/TMS): δ = 1.61 (s, 9 H), 3.55−3.75 (m, 2 H), 4.30−4.63 (m, 1 H), 5.15 (s, 2 H), 5.38 (d, 1 H, *J* = 9), 5.90 (br s, 1 H), 7.38 (s, 5 H).

tert-Butyl (*S*,*Z*)-2-Benzyloxycarbonylamino-5-*tert*-butyldimethylsiloxy-4-hydroxy-2-pentenoate (6):

To a solution of **5** (1.97 g, 5.8 mmol) in CH_2Cl_2 (30 mL) is added 4-dimethylaminopyridine (DMAP, 100 mg) and Et_3N (0.71 g, 7 mmol). After flushing with N_2 *tert*-butylchlorodimethylsilane (1 g, 6.5 mmol) is added and the mixture is stirred under N_2 overnight. The mixture is washed with 1 N aq $KHSO_4$ (20 mL) and sat. aq NaCl (10 mL), dried ($MgSO_4$) and evaporated *in vacuo*. The residue is purified by silica gel chromatography with petroleum ether (bp 40−60 °C)/EtOAc (8:2). Thereby the *E*-isomer (7%) is separated off and the pure *Z*-isomer is isolated; yield: 2.38 g (91%); $[\alpha]_D^{20}$ +14.0° (*c* = 1.96, $CHCl_3$).

$C_{23}H_{37}NO_6Si$	calc.	C 61.17	H 8.26	N 3.10
(451.6)	found	61.31	8.31	3.01

^1H NMR (250 MHz, $CDCl_3$): δ = 0.09 (s, 6 H), 0.91 (s, 9 H), 1.49 (s, 9 H), 3.58−3.72 (m, 2 H), 4.46 (dt, 1 H, J_1 = 8.5, J_2 = 5.4), 5.15 (s, 2 H), 6.33 (d, 1 H, *J* = 8.5), 6.82 (s, 1 H), 7.37 (s, 5 H).

tert-Butyl (2*S*,4*S*)-2-Benzyloxycarbonylamino-5-*tert*-butyldimethylsiloxy-4-hydroxypentanoate (7):

A solution of **6** (1.8 g, 4 mmol) in MeOH (50 mL) containing (*R*,*R*)-$[Rh(1.5\text{-}COD)(DIPAMP)]^+ BF_4^-$ (20 mg) is hydrogenated (3 bar) at r.t. overnight. The mixture is evaporated and the residue chromatographed on silica gel with petroleum ether (bp 40−60 °C)/EtOAc (7:3); yield: 1.79 g (99%); $[\alpha]_D^{20}$ −1.1° (*c* = 0.93, $CHCl_3$).

$C_{23}H_{39}NO_6Si$	calc.	C 60.90	H 8.66	N 3.09
(453.7)	found	60.95	8.78	2.94

^1H NMR (250 MHz, $CDCl_3$): δ = 0.07 (s, 6 H), 0.90 (s, 9 H), 1.47 (s, 9 H), 1.60−1.90 (m, 2 H), 3.52−3.56 (m, 2 H), 3.57−3.80 (m, 1 H), 4.40−4.52 (m, 1 H), 5.12 (s, 2 H), 5.82 (d, 1 H, *J* = 8), 7.36 (s, 5 H).

^{13}C NMR (64 MHz/$CDCl_3$): 171.54, 156.73, 136.29, 128.51, 128.14, 82.17, 68.71, 67.03, 52.07, 36.28, 27.99, 25.89, 18.32, −5.35.

tert-Butyl (*S*)-2-Benzyloxycarbonylamino-5-*tert*-butyldimethylsiloxy-4-oxopentanoate (8):

A solution of oxalyl chloride (0.23 mL, 2.5 mmol) in CH_2Cl_2 (6 mL) is cooled to −55 °C and DMSO (0.39 mL, 5 mmol) is added. The mixture is stirred for 2 min, then the alcohol **7** (1 g, 2.2 mmol) in CH_2Cl_2 (3 mL) is added and stirring is continued for 15 min. To this stirred mixture is added Et_3N (1.54 mL, 11 mmol). After 5 min the mixture is allowed to come to r.t. Next H_2O (10 mL) is added and the aqueous layer is extracted with CH_2Cl_2 (2 × 5 mL). The combined organic layers are washed with sat. aq NaCl, dried ($MgSO_4$) and evaporated. Chromatography on silica gel with petroleum ether (bp 40−60 °C/EtOAc (7:3) gives pure **8**; yield: 0.96 g (97%); $[\alpha]_D^{20}$ +15.2° (*c* = 2.0 $CHCl_3$).

$C_{23}H_{37}NO_6Si$	calc.	C 61.17	H 8.26	N 3.10
(451.6)	found	61.35	8.39	2.92

^1H NMR (250 MHz, $CDCl_3$): δ = 0.08 (s, 6 H), 0.92 (s, 9 H), 1.43 (s, 9 H), 3.03 (dd, 1 H, J_1 = 18.4, J_2 = 4.2), 3.21 (dd, 1 H, J_1 = 18.4, J_2 = 4.2), 4.14 (s, 2 H), 4.52 (dt, 1 H, J_1 = 8.6, J_2 = 4.2), 5.10 (s, 2 H), 5.71 (d, 1 H, *J* = 8.6), 7.34 (s, 5 H).

tert-Butyl (*S*)-2-Benzyloxycarbonylamino-5-hydroxy-4-oxopentanoate (9):

A solution of **8** (0.52 g, 1.15 mmol) in MeCN (9 mL) is treated with hydrogen fluoride-pyridine (0.285 g, 2.9 mmol) and stirred at r.t. for 4 h. Then EtOAc (50 mL) is added and the mixture is washed successively with 1 N aq $KHSO_4$, sat. aq $NaHCO_3$, dried ($MgSO_4$) and purified by chromatography on silica gel (petroleum ether (bp 40−60 °C)/EtOAc, 1:1); yield: 0.37 g (95%); $[\alpha]_D^{20}$ +21.4° (*c* = 2.1, $CHCl_3$).

$C_{17}H_{23}NO_6$	calc.	C 60.52	H 6.87	N 4.14
(337.4)	found	60.28	6.96	4.08

^1H NMR (250 MHz, $CDCl_3$/TMS): δ = 1.43 (s, 9 H), 2.90−3.10 (m, 2 H), 4.24 (s, 2 H), 4.45−4.6 (m, 1 H), 5.10 (s, 1 H), 5.72 (d, 1 H, *J* = 7.6), 7.35 (s, 5 H).

5-Hydroxy-4-oxo-L-norvaline (RI-331) (1):

A mixture of **9** (210 mg, 0.6 mmol) and TFA (2 mL) is stirred at 0 °C for 4 h. The TFA is evaporated *in vacuo* and the solution of the residue in MeOH and 10% AcOH (5:1, 18 mL) is hydrogenated at 3 bar in the presence of Pd-C (30 mg, 5%) over 1 h. The catalyst is filtered off and the solvent evaporated *in vacuo*. The oily residue is dissolved in H_2O (1 mL) and treated with EtOH (3 mL) yielding crude (−)-HON which is recrystallized from H_2O/acetone; yield: 55 mg (62%); $[\alpha]_D^{20}$ −8.2° (*c* = 1.53, H_2O) [Lit. 4 reports no definite mp; $[\alpha]_D^{17}$ −8.2° (*c* = 3.4, H_2O)]

$C_5H_9NO_4$	calc.	C 40.82	H 6.17	9 9.52
(147.1)	found	40.56	6.18	9.38

^1H NMR (250 MHz, D_2O): δ = 3.0−3.25 (m, 2 H), 4.08 (dd, 1 H, *J* = 6.9, 4.6), 4.37 (d, 1 H, *J* = 19.2), 4.46 (d, 1 H, *J* = 19.2).

(*R*)-3-Benzyloxycarbonyl-5-[(*R*)-2-(*tert*-butoxycarbonylamino)-2-(methoxycarbonyl)ethyl]-2,2-dimethyl-1,3-oxazolidine (11):

A solution of (*R*)-3-benzyloxycarbonyl-5-[(*Z*)-2-(*tert*-butoxycarbonylamino)-2-(methoxycarbonyl)vinyl]-2,2-dimethyl-1,3-oxazolidine **10** (4.9 g, 11.3 mmol) in MeOH (90 mL) containing (*S*,*S*)-$[Rh(1.5\text{-}COD)(DIPAMP)]^+ BF_4^-$ (20 mg) is hydrogenated (3 bar) at r.t. overnight. After evaporation the residual diastereomeric mixture (*R*,*R*/*R*,*S*, 8:2) is separated by chromatography on silica gel with petroleum ether (bp 40−60 °C)/EtOAc (7:3); yield: 3.9 g (79%); $[\alpha]_D^{20}$ −14.5° (*c* = 0.94, $CHCl_3$).

$C_{22}H_{32}N_2O_7$	calc.	C 60.54	H 7.34	N 6.42
(436.5)	found	60.34	7.35	6.35

^1H NMR (250 MHz, $CDCl_3$/TMS): δ = 1.43 (s, 9 H), 1.52 (s, 3 H) 1.61 (s, 3 H), 1.90−2.20 (m, 2 H), 3.13 (t, 1 H, *J* = 9.4), 3.74 (s, 3 H), 3.70−3.85 (m, 1 H), 4.10−4.25 (m, 1 H), 4.40−4.60 (br s, 1 H), 5.00−5.20 (m, 2 H), 5.20−5.40 (br s, 1 H), 7.35 (s, 5 H)

^{13}C NMR (63 MHz, $CDCl_3$): δ = 172.57, 155.34, 152.11, 136.55, 128.52, 128.05, 127.94, 94.12, 80.02, 71.36, 66.56, 52.40, 51.40, 50.44, 35.54, 28.27, 26.17, 24.16.

(*R*)-3-Benzyloxycarbonyl-5-[(*R*)-2-(*tert*-butoxycarbonylamino)-2-carboxyethyl]-2,2-dimethyl-1,3-oxazolidine (12):

To a solution of **11** (2.97 g, 6.8 mmol) in dioxane (30 mL) and H_2O (5 mL) is added dropwise 1 N aq LiOH (6.8 mL). When saponification is complete (TLC control), dioxane is distilled off, and the remaining water solution is washed with Et_2O (20 mL). The water layer is acidified and extracted with EtOAc (3 × 30 mL). The combined layers are dried ($MgSO_4$) and evaporated *in vacuo*; yield: 2.85 g (100 %); $[\alpha]_D^{20}$ − 18.8° (c = 1.27, $CHCl_3$).

$C_{21}H_{30}N_2O_7$	calc.	C 59.70	H 7.16	N 6.63
	found	59.36	7.30	6.43

^{1}H NMR (250 MHz, $CDCl_3$/TMS): δ = 1.44 (s, 9 H), 1.53 (s, 3 H), 1.62 (s, 3 H), 1.90–2.20 (m, 2 H), 3.15 (t, 1 H, J = 9.1), 3.70–3.85 (m, 1 H), 4.20–4.35 (br s, 1 H), 4.40–4.60 (br s, 1 H), 5.05–5.20 (m, 2 H), 5.45–5.60 (br s, 1 H), 7.35 (s, 5 H).

(*R*)-3-Benzyloxycarbonyl-5-[(*R*)-2-(*tert*-butoxycarbonylamino)-3-(methoxycarbonyl)propyl]-2,2-dimethyl-1,3-oxazolidine (13):

To a solution of **12** (1.74 g, 4.1 mmol) in THF (10 mL) is added Et_3N (0.42 g, 4.2 mmol) and isobutyl chloroformate (6.6 g, 4.4 mmol) at − 10 °C. After stirring for 10 min, the precipitate is filtered off and the solution treated with a solution of CH_2N_2 in Et_2O (0.2 N, 40 mL). Stirring is continued at 0 °C for 2 h and the mixture is stored overnight in the fridge. THF is distilled off, the residue dissolved in EtOAc (50 mL) and washed with sat. aq $NaHCO_3$ (30 mL) and H_2O (20 mL). The organic layer is dried ($MgSO_4$) and evaporated. The residue is dissolved in MeOH (15 mL) and treated with a solution of silver benzoate in Et_3N (0.1 g in 2 mL). After N_2 evolution ceased the mixture is heated for 10 min at 60 °C, filtered and evaporated. The residue is dissolved in EtOAc (50 mL), washed with 1 N aq $KHSO_4$ (30 mL) and sat. aq $NaHCO_3$, dried ($MgSO_4$) and purified by chromatography on silica gel using petroleum ether (bp 40–60 °C)/EtOAc (7:3) as eluent; yield: 1.39 g ((75 %); $[\alpha]_D^{20}$ − 4.0° (c = 1.37, $CHCl_3$).

$C_{23}H_{34}N_2O_7$	calc.	C 61.32	H 7.61	N 6.22
(450.5)	found	61.22	7.58	6.09

^{1}H NMR (250 MHz, $CDCl_3$/TMS): δ = 1.40 (s, 9 H), 1.52 (s, 3 H), 1.59 (s, 3 H), 1.65–2.00 (m, 2 H), 2.61 (d, 2 H, J = 5.5), 3.10 (d, 1 H, J = 9.6), 3.68 (s, 3 H), 3.60–3.85 (m, 1 H), 4.00–4.20 (m, 2 H), 5.00–5.10 (m, 2 H), 5.10–5.30 (br s, 1 H), 7.35 (s, 5 H).

^{13}C NMR (63 MHz, $CDCl_3$): δ = 172.07, 155,15, 152.5, 136.66, 128.54, 127.96, 93.76, 79.46, 71.80, 66.54, 51.72, 50.67, 45.40, 38.95, 37.46, 28.36, 26.18, 24.15.

(*R*)-3-Benzyloxycarbonyl-5-[(*R*)-2-(*tert*-butoxycarbonylamino)-3-carboxypropyl]-2,2-dimethyl-1,3-oxazolidine (14):

A solution of **13** (1.17 g, 2.7 mmol) in dioxane (10 mL) and H_2O (3 mL) is treated with 1 N aq LiOH (2.7 mL). When saponification is complete (TLC control) dioxane is distilled off and the remaining water solution is washed with Et_2O (10 mL). The water layer is acidified and extracted with EtOAc (3 × 20 mL). The combined organic layers are dried ($MgSO_4$) and evaporated *in vacuo*; yield: 1.16 g (100 %); $[\alpha]_D^{20}$ − 8.5 (c = 1.7, $CHCl_3$).

^{1}H NMR (250 MHz, $CDCl_3$/TMS): δ = 1.44 (s, 9 H), 1.52 (s, 3 H), 1.60 (s, 3 H), 1.70–2.15 (m, 2 H), 2.66 (d, 2 H, J = 5.6), 3.11 (t, 1 H, J = 4.6), 3.65–3.90 (m, 1 H), 4.00–4.25 (m, 2 H), 5.05–5.40 (m, 3 H), 7.35 (s, 5 H).

(2-*tert*-Butoxycarbonyl-1-methylhydrazino)acetic Acid (15):

A solution of methylhydrazine (36 g, 0.76 mol) in H_2O (200 mL) is cooled to 0 °C and chloroacetic acid (13.5 g, 0.143 mol) is added over a period of 30 min. The mixture is stirred for 3 d. Then NaOH (11.6 g, 0.29 mol) is added and the solvent is evaporated *in vacuo*. The residue is dissolved in H_2O (70 mL), NaOH (5.7 g, 0.14 mol) is added and the mixture cooled to 0 °C. The di-*tert*-butyl dicarbonate (30 g, 0.14 mol) in dioxane (50 mL) is added and the mixture warmed up to r. t. overnight. After evaporation of dioxane *in vacuo*, the water layer is washed with Et_2O (50 mL), acidified to pH 2 and extracted with EtOAc (3 × 50 mL). The organic layers are combined, dried ($MgSO_4$) and evaporated *in vacuo* yielding a yellowish solid which is recrystallized from Et_2O/petroleum ether (bp 55 °C); yield: 15.5 g (53 %), mp 119 °C (dec).

$C_8H_{16}N_2O_4$	calc.	C 47.05	H 7.90	N 13.72
(204.2)	found	47.09	8.02	14.01

^{1}H NMR (80 MHz, $CDCl_3$/TMS): δ = 1.40 (s, 9 H), 2.60 (s, 3 H), 3.48 (s, 2 H), 7.89 (br s, 1 H)

Benzyl (2-*tert*-Butoxycarbonyl-1-methylhydrazino)acetate (16):

A solution of benzyl alcohol (0.54 g, 5 mmol), DMAP (62 mg) and DCC (1.14 g, 5.5 mmol) in CH_2Cl_2 (10 mL) is stirred at − 10 °C for 15 min. Then **15** (1.02 g, 5 mmol) in CH_2Cl_2 (10 mL) is added and the mixture warmed up to r. t. overnight. The solvent is evaporated *in vacuo* and the residue dissolved in Et_2O (20 mL) and filtered. The solution is washed with sat. aq $NaHCO_3$, dried ($MgSO_4$) evaporated *in vacuo* and the resulting white solid is crystallized from petroleum ether (bp 40–60 °C); yield: 1.05 g (71 %); mp 66 °C

$C_{15}H_{22}N_2O_4$	calc.	C 61.21	H 7.53	N 9.60
(294.4)	found	61.33	7.66	9.52

^{1}H NMR (250 MHz, $CDCl_3$/TMS): δ = 1.45 (s, 9 H), 2.77 (s, 3 H) 3.73 (s, 2 H), 5.17 (s, 2 H), 6.69 (br s, 1 H), 7.36 (s, 5 H)

(*R*)-3-Benzyloxycarbonyl-5-[(*R*)-4-(2-benzyloxycarbonyl-2-methylhydrazino)-2-*tert*-butoxycarbonylamino-4-oxobutyl]-2,2-dimethyl-1,3-oxazolidine (18):

To a solution of **14** (0.35 g, 0.8 mmol) in THF (10 mL) is added Et_3N (0.34 g, 3 mmol) at − 20 °C. Then ethyl chloroformate (0.11 g, 1 mmol) is added and stirring continued for 10 min. To this mixture is added benzyl (1-methylhydrazino)acetate as the hydro trifluoroacetate **17** (345 mg, 1.12 mmol), prepared from **16** with CF_3CO_2H) in THF (10 mL). The mixture is warmed up to r. t. overnight, THF is distilled off and the residue dissolved in EtOAc (20 mL) and filtered. This solution is washed with 1 N aq $KHSO_4$ (10 mL) and sat. aq $NaHCO_3$ (10 mL), dried ($MgSO_4$) and evaporated *in vacuo*. Chromatography on silica gel using EtOAc as eluent gives **18** as a colorless oil; yield: 0.41 g (84 %); $[\alpha]_D^{20}$ − 3.8° (c = 0.9, $CHCl_3$);

HRMS: m/z, $C_{32}H_{44}N_4O_8$ calc.: (M^+) 612.3148; found: 612.312.

^{1}H NMR (250 MHz, $CDCl_3$/TMS): δ = 1.30–2.10 (m, 18 H), 2.37–2.42 (m, 1 H), 2.72, 2.78 (each s, total 3 H), 3.00–3.20 (m, 1 H), 3.50–3.80 (m, 1 H), 3.73, 3.74 (each s, total 2 H), 3.85–4.30 (m, 2 H), 5.09 (s, 2 H), 5.16 (s, 2 H), 5.50–5.70 (m, 1 H), 7.30–7.40 (m, 10 H), 7.87 (s, 1 H).

MS: m/z (%) = 613 (M^+ + 1, 1.0), 612 (M^+, 2.6), 91 (100).

Benzyl [(3*R*,5*R*)-6-Benzyloxycarbonylamino-3-*tert*-butoxycarbonylamino-5-hydroxyhexanoyl-1-methylhydrazino]-acetate (19):

A solution of **18** (210 mg, 0.34 mmol) in AcOH 50 % (10 mL) is stirred at r. t. for 24 h. Then H_2O (10 mL) is added. The mixture is neutralized with $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 15 mL). The organic layers are combined, dried ($MgSO_4$) and evaporated *in vacuo*. Pure **19** is obtained by chromatography on silica gel using EtOAc as eluent; yield: 130 mg (67 %); $[\alpha]_D^{20}$ + 6.8° (c = 2.4, $CHCl_3$).

HRMS: m/z, $C_{29}H_{40}N_4O_8$ calc.: (M^+) 572.2836; found: 572.2852.

^{1}H NMR (250 MHz, $CDCl_3$/TMS): δ = 1.42 (s, 9 H), 1.50–1.85 (m, 2 H), 2.00–2.80 (m, 2 H), 2.71, 2.77 (each s, total 3 H), 2.90–3.10 (m, 1 H), 3.30–3.45 (m, 1 H), 3.50–3.75 (m, 1 H), 3.73 (s, 2 H), 4.00–4.20 (m, 1 H), 5.09 (s, 2 H), 5.18 (s, 2 H), 5.30 (br s, 1 H), 5.95 (t, 1 H, J = 9.2), 7.34 (s, 5 H), 7.37 (s, 5 H), 7.78 (s, 1 H).

MS: m/z (%) = 572 (M^+, 0.49), 91 (100).

(+)-Negamycin (2):

A solution of **19** (120 mg, 0.21 mmol) in TFA (2 mL) is stirred for 1 h at 0 °C. The solvent is evaporated *in vacuo* and the residue dissolved in MeOH/10 % AcOH (2:1, 30 mL) then Pd-C (100 mg, 5 %) is added and the mixture is hydrogenated (3 bar) overnight. After filtration from the catalyst the mixture is evaporated *in vacuo*. The residue is dissolved in H_2O (10 mL) and neutralized with aq NH_3 (1 %). This solution is applied to a column of Amberlite CG 50 (NH_4^+-Form). After eluation with H_2O negamycin is eluted with aq NH_3 (0.2 %). Lyophylisation gives pure **2**; yield: 50 mg (96 %); $[\alpha]_D^{20}$ + 2.5° (c = 1.4, H_2O); mp 110–114 °C (dec) [Lit. 11 mp 110–120 °C; $[\alpha]_D^{29}$ + 2.5° (c = 2, H_2O).

^{1}H NMR (250 MHz, D_2O): δ = 1.50–1.80 (m, 2 H), 2.42 (d, 2 H, J = 7.4), 2.62 (s, 3 H), 2.85–3.15 (m, 2 H), 3.39 (s, 2 H), 3.45–3.60 (m, 1 H), 3.95–4.15 (m, 1 H).

This work was supported by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie and BASF AG.

Received: 24 June 1991

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