A Total Synthesis of (+)-Negamycin through Isoxazolidine Allylation

Roderick W. Bates,* Rab'iah Nisha Khanizeman, Hajime Hirao, Yu Shan Tay and Patcharaporn Sae-Lao

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The β-amino acid antibiotic (+)-negamycin has been synthesised in ten steps from epichlorohydrin via Sakurai allylation of an isoxazolidine intermediate. The key allylation reaction proceeded with complete *trans*-selectivity, which is attributed to electrostatic attraction between the chlorine atom and the iminium ion in the Sakurai intermediate.

Negamycin **1** was isolated from the microorganism *Streptomyces purpeofuscus* and shown to have low toxicity, but significant activity against both Gram-positive and Gram-negative bacteria.[1]

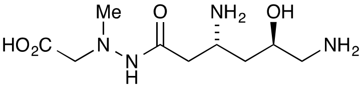
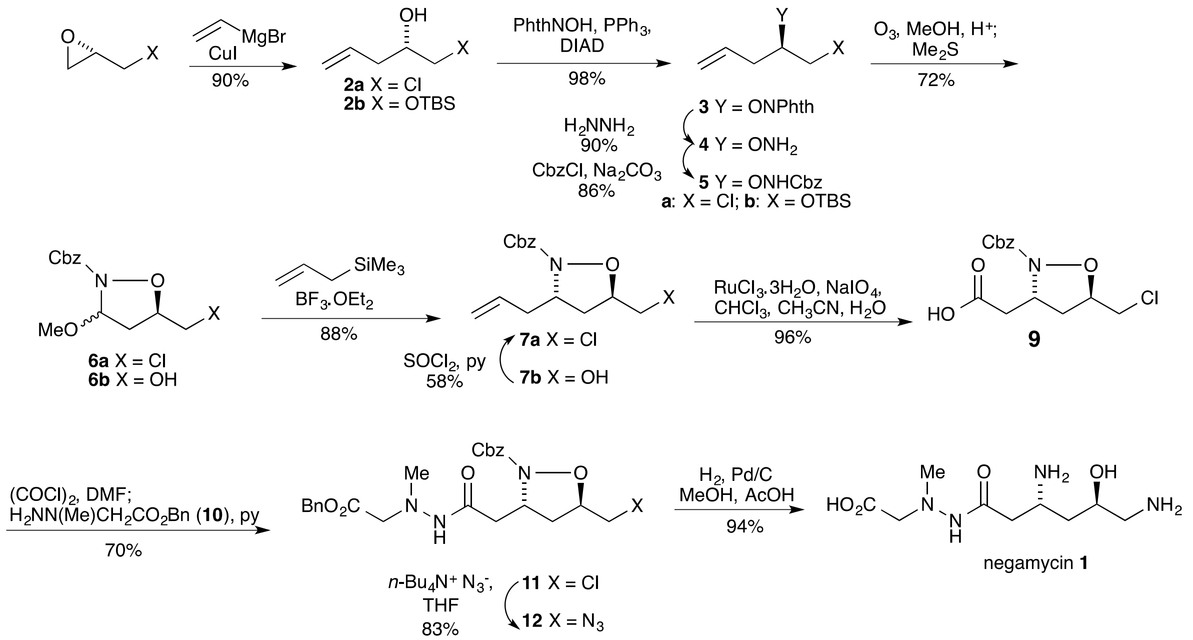


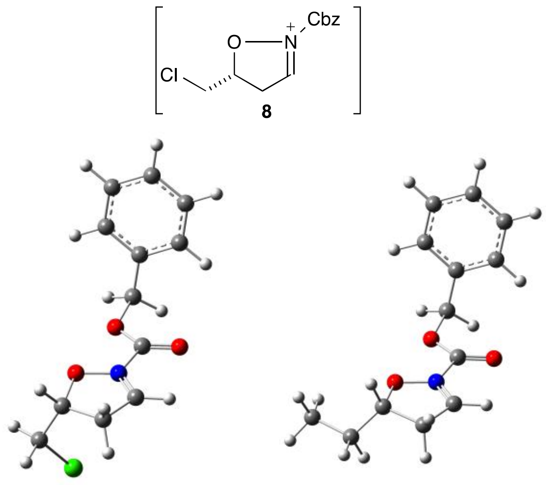
Fig. 1 Negamycin

Given the current dearth of new antibiotics, it remains a molecule of interest. Negamycin has been the subject of a number of syntheses, as have its stereoisomers and analogs.[2] A number of interesting strategies have been employed over the years. One method to generate the required 1,3-*anti* relationship between the hydroxyl and amino groups is to use a 1,3-dipolar cycloaddition to form a 3,5-*trans*-isoxazolidine, followed by cleavage of the *N*,*O*-bond at a later stage. Such reactions often give the *trans*-isoxazolidine as the major stereoisomer. This method was, indeed, employed by Kibayashi.[2e,h] Over the years, this laboratory has reported a number of non-cycloaddition methods for the synthesis of 3,5-*cis*-isoxazolidines, which give *syn*-1,3-aminoalcohol derivatives upon *N*,*O*-bond cleavage.[3] Recently we reported that the Sakurai reaction of *N*-carboalkoxy-3-methoxy isoxazolidines gives 3-allylisoxazolidines with modest selectivity for the *trans* isomer.[4] We considered that applying this method to the synthesis of negamycin would be a useful contribution to the field.

The 5-chloromethyl-3-methoxyisoxazolidine **6a** was prepared with the intention of using the chlorine atom to introduce the terminal amino group at a late stage. Ring opening of (*S*)-*epi*-chlorohydrin with vinyl magnesium bromide, catalysed by copper(I) iodide, gave the alcohol **2**.[5] This compound was subjected to the sequence of Mitsunobu reaction with *N*-hydroxyphthalimide,[6] dephthaloylation and *N*-reprotection, which we have used extensively, to give hydroxylamine **5** in 76% yield over the three steps. A Cbz group was chosen for *N*-protection as we anticipated a global hydrogenation as the final step, as used by others.[2c,d,e,f,g,h,m,p,q,t] The *N*-protected hydroxylamine **5** was subjected to ozonolysis in acidic methanol. This gave the methoxyisoxazolidine **6a** as an inconsequential mixture of diastereoisomers directly in 72% yield after a dimethyl sulfide work-up. To our surprise, allylation of this isoxazolidine under the conditions that we reported earlier gave the Sakurai product **7a** as a single stereoisomer (within the limits of detection by 400 MHz spectroscopy), rather than the *ca* 4:1 mixture anticipated based upon those results.[4] Subsequent conversion to the natural product showed that this was the desired *trans* isomer. We attribute the stereochemical outcome to an electrostatic effect. The Sakurai reaction is presumed to proceed via an iminium ion intermediate **8**. A simple aryl or alkyl substituent at C5 exerts only a modest influence as the conformation of five member rings is poorly defined. In this case, we postulate an electrostatic attraction between the chlorine atom (δ-) and the positive iminium ion that results in highly effective shielding of one face. Indeed, calculation of the conformation of this iminium ion intermediate indicated that the lowest energy conformation has the chlorine atom over the isoxazolidine ring, where it would provide efficient shielding of that face (Figure 2).[7] This conformation was found to be 2.9 kcal/mol lower than the next lowest conformation. In contrast, analogous calculation of the conformation of the corresponding ethyl substituted iminium ion, placed the CH_3 group of the ethyl group in free space, away from the ring, though other conformations were within 1 kcal/mol. This correlates with the modest selectivity observed with isoxazolidines with simple substituents. Whatever the cause, the outcome was very gratifying.[8] Other electronegative atoms would be expected to show the same behavior. The hydroxymethyl isoxazolidine **6b** was prepared in racemic form by an analogous route starting from the TBS ether of glycidol. The two diastereoisomers of **6b** were separable by flash chromatography (though not identified). Treatment of either diastereoisomer with allyltrimethylsilane under the conditions used for the conversion of **6a** to **7a** gave exclusively the *trans*-isoxazolidine **7b**. The *trans* stereochemistry of isoxazolidine **7b** was confirmed by conversion to **7a** on treatment with thionyl chloride and pyridine.



Scheme 1 Negamycin Synthesis



Scheme 2 Calculated lowest energy conformations of iminium ion intermediates with chloromethyl and ethyl substituents.

Oxidative cleavage of alkene **7a** under Sharpless' conditions[9] gave carboxylic acid **9**. It was found that the most effective way to couple the acid with the known hydrazine **10**[2k] was by formation of the acid chloride. Subsequent displacement of the chloride of hydrazide **11** with azide proved troublesome. Attempts to achieve this using sodium azide, with or without addition of a sodium iodide catalyst, in DMF were fruitless, returning starting material, or, on heating, leading to decomposition. On the other hand, use of tetra-*n*-butylammonium azide[10] in THF gave the desired azide **12**, characterized by a strong IR absorbance at 2104 cm^{-1}. Global hydrogenation of azide **12**, involving the removal of two protecting groups, unmasking of the amine and cleavage of the *N*,*O*-bond, then gave (+)-negamycin **1**, identical to the natural product: m.p. 114-117 °C (Lit.[1a]: 110 - 120 °C), $[\alpha]_D^{23}$ +2.5 (*c* 2.0, H_2O) (Lit.[1a]: $[a]_D^{29}$: +2.5 (*c* 2.0, H_2O)). The natural product was obtained in 93% yield after purification by ion exchange chromatography. The overall yield is 23% over 10 steps. The completion of the synthesis further illustrates the utility of non-cycloaddition methods for the synthesis of *N*,*O*-heterocycles and, thence, amino alcohols and their derivatives. The observation of the very high stereoselectivity during the Sakurai reaction underlines the importance of subtle electrostatic effects upon the conformation of reactive intermediates.

Experimental

All reactions requiring anhydrous conditions were carried out under a nitrogen atmosphere using oven-dried glassware (120 °C), which was cooled under vacuum. Anhydrous tetrahydrofuran was distilled from sodium metal and benzophenone under nitrogen. Anhydrous dichloromethane was dried by distillation from CaH_2 immediately prior to use under nitrogen. Anhydrous methanol was distilled from activated magnesium under nitrogen. All other solvents and reagents were used as received. Flash chromatography was carried out on silica gel, 230-400 Mesh. ^1H NMR spectra were recorded at 300, 400 or 500 MHz in $CDCl_3$ solutions. ^{13}C NMR spectra were recorded at the corresponding frequency on the same instruments at 75, 100 or 125 MHz. Chemical shifts are recorded in ppm and coupling constants are recorded in Hz. Optical rotations are given with units of 10^{-1}degcm^{2}g^{-1}. Rotations were measured at a wavelength of 589 nm. Melting points are uncorrected.

(*S*)-1-chloropent-4-en-2-ol (2): Vinyl magnesium bromide was prepared by dropwise addition of vinyl bromide (10 mL) to a stirring suspension of magnesium turnings (0.73 g, 30.0 mmol) in anhydrous tetrahydrofuran (35.0 mL). The mixture was heated at reflux for 1 hour. The vinyl magnesium bromide was added dropwise to a cooled (-68 °C) solution of *(S)*-epichlorohydrin (0.85 mL, 10.81 mmol) containing copper(I) iodide (0.21 g, 1.08 mmol) in anhydrous diethyl ether (45.0 mL). The mixture was stirred for 1 hour at -68 °C. The mixture was quenched with saturated aqueous ammonium chloride and allowed to warm to room temperature. The layers were separated and the aqueous layer was extracted with diethyl ether (3 × 10 mL). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulphate, filtered through celite and concentrated *in vacuo* to give alcohol (**2**) (1.17 g, 90 %) as a colorless clear oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.82 (1H, ddt, J = 17.1, 7.1, 3.2 Hz), 5.17 (2H, dd, J = 13.1, 1.4 Hz), 3.90-3.85 (1H, m) 3.63 (1H, dd, J = 11.2, 4.1 Hz), 3.51 (1H, dd, J = 11.2, 6.8 Hz), 2.39-2.31 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 133.2, 118.6, 70.5, 49.3, 38.6; MS (ESI) *m/z* 121 $[M+H]^+$. The obtained data are consistent with literature values.[5]

(*R*)-2-(1-chloropent-4-en-2-yloxy)isoindoline-1,3-dione (3): *(S)*-1-Chloropent-4-en-2-ol, (**2**) (2.0 g, 16.60 mmol) in anhydrous tetrahydrofuran (50 mL) was added to a mixture of *N*-hydroxyphthalimide (3.25 g, 19.90 mmol) and triphenylphosphine (6.53 g, 24.70 mmol). The mixture was then cooled to 0 °C and diisopropyl azodicarboxylate (5.8 mL, 29.70 mmol) in anhydrous tetrahydrofuran (5 mL) was added dropwise with the temperature maintained at 0 °C until complete addition. The mixture was warmed to room temperature and stirred overnight. The solvent was removed *in vacuo*. The residue was purified by dry-loading flash chromatography on silica gel eluting with (15 % EtOAc / Hexane) to give hydroxylamine (**3**) (4.30 g, 98 %) as a colorless solid. Mp = 60- 71 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90-7.76 (4H, m), 5.96 (1H, ddt, J = 17.4, 7.3, 3.2 Hz), 5.23 (1H, dd, J = 16.7, 1.4 Hz), 5.21 (1H, dd, J = 11.7, 1.8 Hz), 4.47-4.44 (1H, m), 3.78 (2H, dd, J = 11.9, 4.6 Hz), 2.73 (1H, ddd, J = 14.7, 6.4, 6.0 Hz), 2.63 (1H, ddd, J = 16.1, 11.2, 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.7, 134.6, 131.8, 128.8, 123.6, 118.9, 86.0, 43.4, 34.9; IR ν_{max} 1790, 1732, 1612, 1188 cm^{-1}; MS (ESI) *m/z* 289 $[M+Na]^+$; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calculated for $C_{13}H_{13}{}^{35}ClNO_3$: 266.0584; found: 266.0580; $[\alpha]_D^{23}$ +8.9 (*c* 0.2, CH_2Cl_2).

(*R*)-O-(1-chloropent-4-en-2-yl)hydroxylamine (4): Hydrazine monohydrate (0.45 mL, 14.2 mmol) was added to a solution of hydroxylamine (**3**) (0.59 g, 2.22 mmol) in dichloromethane (20 mL) at 0 °C. The mixture was warmed to room temperature, stirred for 15 minutes, filtered through celite, and concentrated *in vacuo* to give hydroxylamine (**4**) (0.27 g, 90 %) as a colorless oil which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.80 (1H, ddt, J = 17.4, 7.3, 3.2 Hz), 5.17 (1H, dd, J = 3.2, 1.4 Hz), 5.13 (1H, dd, J = 3.9, 1.4 Hz), 3.83-3.71 (1H, m), 3.64 (2H, dd, J = 11.4, 4.6 Hz), 2.42 (1H, ddd, J = 11.5, 7.6, 1.4 Hz), 2.36 (1H, ddd, J = 11.8, 7.1, 1.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 134.1, 117.6, 80.4, 48.9, 38.6; IR ν_{max} 3306, 2982, 1043 cm^{-1}; MS (ESI) *m/z* 158 $[M+Na]^+$; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calculated for $C_5H_{11}{}^{35}ClNO$: 136.0529; found: 136.0528; $[\alpha]_D^{21}$ +3.0 (*c* 1.0, CH_2Cl_2).

(*R*)-benzyl 1-chloropent-4-en-2-yloxycarbamate (5): Benzyl chloroformate (1.67 mL, 12.30 mmol) was added to a solution of (**4**) (1.25 g, 10.30 mmol) in CH_2Cl_2 : H_2O (1:1) (78 mL) containing anhydrous sodium carbonate (1.31 g, 12.30 mmol) at 0 °C. The mixture was stirred at room temperature for 3 hours. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with (25 % EtOAc / Hexane) to give hydroxylamine (**5**) (2.39 g, 86 %) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40-7.34 (5H, m), 5.81 (1H, ddt, J = 17.4, 7.3, 3.2 Hz), 5.18 (2H, s), 5.15 (2H, dd, J = 5.0, 1.8 Hz), 4.15-4.04 (1H, m), 3.72 (1H, dd, J = 11.9, 5.0 Hz), 3.63 (1H, dd, J = 7.5, 4.6 Hz), 2.49 (2H, ddd, J = 7.5, 3.4, 1.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.8, 135.4, 132.9, 128.7, 128.6, 128.5, 118.6, 83.9, 67.9, 43.9, 34.8; IR ν_{max} 3083, 2982, 1707, 1647, 754, 698 cm^{-1}; MS (ESI) *m/z* 270 $[M+H]^+$; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calculated for $C_{13}H_{16}{}^{35}ClNO_3Na$: 292.0716; found: 292.0713; $[\alpha]_D^{22}$ + 27.8 (*c* 0.3, CH_2Cl_2).

(5*R*)-2-benzyloxycarbonyl-5-chloromethyl-3-methoxyisoxazolidine (6a): Amberlyst-15 (66.0 mg, 0.48 mmol) was added to a solution of (**5**) (2.56 g, 9.50 mmol) in methanol (52.0 mL) at -78 °C. The temperature was maintained at -78 °C while a mixture of ozone and oxygen was bubbled through the solution until a blue color was observed. Dimethyl sulfide (8.40 mL) was added to the solution and it was stirred overnight, then filtered and concentrated *in vacuo*. Water was added, and the mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulphate, filtered through celite and concentrated *in vacuo* to give isoxazolidine (**6a**) (1.95 g, 72 %) as a colorless clear oil and as an inconsequential 1:1 mixture of diastereomers.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40-7.31 (5H, m), 5.47 (1H, dd, J = 6.3, 1.6 Hz), 5.27-5.17 (3H, m), 4.68-4.59 (1H, m), 4.40-4.33 (1H, m), 3.71 (1H, dd, J = 11.4, 5.5 Hz), 3.64 (1H, dd, J = 11.4, 6.8 Hz), 2.58 (1H, ddd, J = 14.8, 6.4, 1.4 Hz), 2.46 (1H, ddd, J = 13.6, 7.3, 1.8 Hz), 2.36 (1H, ddd, J = 14.7, 7.1, 1.4 Hz,), 2.22 (1H, ddd, J = 13.6, 6.7, 1.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.0, 145.7, 135.6, 128.8, 128.7, 128.5, 128.3, 91.2, 90.3, 80.8, 56.2, 56.0, 44.8, 44.7, 43.6, 39.3, 39.1; IR ν_{max} 3034, 2955, 1454, 1741, 7752, 698 cm^{-1}; MS (ESI) *m/z* 309 $[M+Na]^+$; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calculated for $C_{13}H_{16}{}^{35}ClNO_4Na$: 308.0666; found: 308.0667; $[\alpha]_D^{22}$ -10.4 (*c* 8.8, CH_2Cl_2).

(3*S*,5*R*)-3-allyl-2-benzyloxycarbonyl-5-chloromethylisoxazolidine (7a): Boron trifluoride diethyl etherate (1.78 mL, 14.2 mmol) was added dropwise to a solution of (**6a**) (1.56 g, 5.46 mmol) and allyltrimethylsilane (2.61 mL, 16.4 mmol) in anhydrous dichloromethane (80 mL) at -70 °C. The mixture was stirred at -40 °C for 24 h. The mixture was quenched with triethylamine (2.0 mL) at -40 °C, then allowed to warm to room temperature. Water was added, and the mixture was extracted with dichloromethane (3 × 20 mL). The combined organic extracts were washed with brine, dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with (3-4 % EtOAc / Hexane) to give isoxazolidine (**7a**) (1.42 g, 88 %) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41-7.31 (5H, m), 5.78 (1H, ddt, *J* = 12.1, 6.9, 3.7 Hz), 5.23 (1H, d, *J* = 12.4 Hz), 5.16 (1H, d, *J* = 12.4 Hz), 4.48-4.45 (1H, m), 4.38-4.36 (1H, m), 3.54 (1H, dd, *J* = 11.2, 5.5 Hz), 3.29 (1H, dd, *J* = 11.4, 7.8 Hz), 2.47 (2H, ddd, *J* = 16.9, 14.2, 6.8 Hz), 2.34-2.36 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.7, 135.9, 133.8, 128.7, 128.6, 128.5, 118.4, 80.2, 68.2, 58.7, 44.5, 39.1, 36.7; IR ν_{max} 3066, 3032, 2954, 1703, 1641, 993, 918, 754, 698 cm^{-1}; MS (ESI) *m/z* 297 $[M+H]^+$; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calculated for $C_{15}H_{18}{}^{35}ClNO_3Na$: 318.0873; found: 318.0878; $[\alpha]_D^{22}$ +93.5 (*c* 3.5, CH_2Cl_2).

2-((3*R*, 5*R*)-2-benzyloxycarbonyl-5-(chloromethyl)isoxazolidinyl)acetic acid (9): Ruthenium trichloride (0.91 mg, 0.00440 mmol) was added to a solution of isoxazolidine (**7a**) (0.060 g, 0.20 mmol), and sodium metaperiodate (0.175 g, 0.82 mmol) in chloroform (0.40 mL), acetonitrile (0.40 mL), water (0.60 mL). The mixture was stirred vigorously for 2 h at room temperature. Dichloromethane (2 mL) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 × 10 mL). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give carboxylic acid (**9**) (0.06 g, 96 %) as a bright orange-yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.41 (5H, m), 5.26 (1H, d, *J* = 12.1 Hz), 5.20 (1H, d, *J* = 12.1 Hz), 4.75-4.68 (1H, m), 4.54-4.48 (1H, m), 3.56 (1H, dd, *J* = 11.7, 5.3 Hz), 3.33 (1H, dd, *J* = 11.2, 7.5 Hz) 2.90 (1H, dd, *J* = 16.9, 5.5 Hz), 2.60 (1H, dd, *J* = 16.7, 8.5 Hz), 2.48 (1H, ddd, *J* = 17.6, 8.2, 4.6 Hz), 2.33 (1H, ddd, *J* = 17.6, 8.5, 4.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.9, 157.4, 135.6, 128.8, 128.7, 128.5, 80.1, 68.6, 55.9, 44.4, 39.1, 37.7; IR ν_{max} 2961, 1732, 1714, 1454, 1423, 752, 698 cm^{-1}; MS (ESI) *m/z* 336 $[M+Na]^+$; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calculated for $C_{14}H_{16}{}^{35}ClNO_5Na$: 336.0615; found: 336.0612; $[\alpha]_D^{22}$ +13.1 (*c* 2.0, CH_2Cl_2).

(3*R*,5*R*)-benzyl 3-(2-(2-(2-(benzyloxy)-2-oxoethyl)-2-methylhydrazinyl)-2-oxoethyl)-5-(chloromethyl)isoxazolidine-2-carboxylate (11): Dimethylformamide (0.50 mL) was added to a solution of acid (**9**) (1.52 g, 4.84 mmol) and oxalyl chloride (0.741 mL, 8.65 mmol) in dichloromethane (15 mL). The mixture was stirred for 0.5 h. The volatiles were removed *in vacuo* and the residue was taken up in dichloromethane (83 mL). Hydrazine (**10**) (1.12 g, 5.60 mmol) followed by pyridine (0.700 mL, 8.65 mmol) were added and the mixture was stirred for 24 h. The volatiles were removed *in vacuo* and the residue was purified by flash chromatography on silica gel eluting with (30 % MeOH / EtOAc) to give hydrazide (**11**) (1.65 g, 70 %) as a dark brown oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.35 (10H, m), 5.20-5.14 (4H, m), 4.74-4.68 (1H, m), 4.49-4.47 (1H, m), 3.73 (1H, d, *J* = 18.0 Hz), 3.68 (1H, d, *J* = 18.0 Hz), 3.52 (1H, dd, *J* = 12.1, 5.7 Hz), 3.32 (1H, dd, *J* = 11.4, 8.2 Hz), 2.74 (3H, s), 2.47 (1H, dd, *J* = 14.3, 6.4 Hz), 2.32 (1H, dd, *J* = 14.3, 6.4 Hz), 2.39 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 167.8, 157.4, 135.8, 135.6, 135.4, 128.9, 128.8, 128.7, 128.6, 80.3, 68.5, 66.7, 57.6, 44.5, 39.8, 37.2; IR ν_{max} 2955, 1714, 1668, 1385 cm^{-1}; MS (ESI) *m/z* 512 $[M+Na]^+$; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calculated for $C_{24}H_{28}{}^{35}ClN_3O_6Na$: 512.1564; found: 512.1560; $[\alpha]_D^{22}$ +42.5 (*c* 11.3, CH_2Cl_2).

Benzyl 2-((3*R*, 5*R*)- 5-azidomethyl-2-(benzyloxycarbonyl)isoxazolidinyl)acetate (12): *n*-$Bu_4N^+N_3^-$ (0.0820 g, 0.330 mmol) was added to a stirred solution of chloride (**11**) (0.080 g, 0.163 mmol) in tetrahydrofuran (5 mL) at 25 °C. The mixture was heated at reflux for 24 h. The solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel eluting with (10 % MeOH / EtOAc) to give azide (**12**), (0.067 g, 83 %) as a dark brown oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.33 (10H, m), 5.26-5.14 (4H, m), 4.70-4.65 (1H, m), 4.41-4.17 (1H, m), 3.73 (1H, d, *J* = 18.0 Hz), 3.68 (1H, d, *J* = 18.0 Hz), 3.32 (1H, dd, *J* = 7.1, 4.6 Hz), 3.15 (1H, dd, *J* = 8.7, 4.6 Hz), 2.75 (3H, s), 2.45 (1H, dd, *J* = 14.2, 6.4 Hz), 2.32 (1H, dd, *J* = 14.4, 6.6 Hz), 1.64 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 169, 155, 157.4, 135.6, 135.4, 135.2, 128.9, 128.8, 128.7, 128.6, 80.0, 68.6, 62.6, 55.9, 48.9, 44.4, 39.1, 37.7; IR ν_{max} 3242, 3065, 3034, 2957, 2104, 1745, 1693, 1497, 745, 698; MS (ESI) *m/z* 519 $[M+Na]^+$; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calculated for $C_{24}H_{28}{}^{35}ClN_6O_6Na$: 519.1968; found: 519.1968; $[\alpha]_D^{22}$ +23.3 (*c* 2.5, CH_2Cl_2).

(+)-Negamycin (1): A solution of azide (**12**) (24.1 mg, 0.0485 mmol) in a 2 : 1 mixture of methanol and 5 % aqueous acetic acid (2.28 mL) containing 10 % palladium on charcoal (4 mg) in a Fisher-Porter tube was placed under H_2 (40 psi) and stirred at room temperature for 24 h. The mixture was then filtered through a wet celite pad washing with water. The volatiles were removed *in vacuo* and the residue was purified by ion-exchange chromatography on amberlite CG-50 (NH_4^+ resin), eluting with water followed by 0.5 % NH_4OH (aq). Ninhydrin-active fractions were collected and concentrated *in vacuo* to give (+)-Negamycin (**1**) (11.3 mg, 94 %) as colorless powder.

Mp = 114 - 117 °C (lit mp: 110 - 120 °C)[1a]; ^{1}H NMR (400 MHz, D_2O): δ 3.84-3.79 (1H, m), 3.27-3.22 (1H, m), 3.23 (2H, s), 2.85 (1H, dd, *J* = 13.2, 3.5 Hz), 2.70 (1H, dd, *J* = 13.2, 9.0 Hz), 2.46 (3H, s), 2.40 (1H, dd, *J* = 14.6, 6.4 Hz), 2.15 (1H, dd, *J* = 14.5, 7.3 Hz), 1.48-1.37 (2H, m); ^{13}C NMR (100 MHz, D_2O): δ 177.1, 170.9, 65.6, 61.0, 45.2, 44.9, 43.8, 41.2, 39.6; $[\alpha]_D^{23}$: +2.5 (*c* 2.0, H_2O) lit: $[\alpha]_D^{29}$: +2.5 (*c* 2.0, H_2O).[1a]

Tetrabutylammonium Azide:[11] A solution of NaN_3 (1.00 g, 15.4 mmol) in water (2.3 mL) was added to tetrabutylammonium hydroxide (40% aqueous solution, 2.00 g, 7.69 mmol). Dichloromethane (12 mL) was added, and the organic layer was separated. The aqueous layer was extracted with dichloromethane (3 × 4 mL). The combined organic layers were dried over anhydrous magnesium sulphate, filtered and concentrated to give the salt as a white crystalline solid (2.10 g, 96 %).

IR v_{max} 2014 cm^{-1}.

Synthesis of the Hydroxy Analogs: In all cases, the procedures used were the same as for the chloro compounds. All compounds in the hydroxy series are racemic.

(±)-1-(*t*-Butyldimethylsilyloxy)pent-4-en-2-ol (2b)

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.84 (1H, ddt, J = 17.4, 10.1, 7.2 Hz), 5.07-5.14 (2H, m), 3.70 (1H, m), 3.63 (1H, dd, J = 10.1, 3.7 Hz), 3.46 (1H, dd, J = 10.1, 6.9 Hz), 2.40 (1H, d, J = 3.6 Hz), 2.24 (2H, m), 0.90 (9H, s), 0.07 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 134.4, 117.3, 71.1, 66.5, 37.6, 25.7, 25.6, 18.2, -5.4; IR v_{max} 3423, 3076, 2954, 2928, 1641, 1258, 837, 777 cm^{-1}; MS (ESI) *m/z* 217 [M+H]$^+$; HRMS (ESI-TOF) *m/z* [M+H]$^+$ calculated for $C_{11}H_{24}O_2Si$: 217.1624; found: 217.1621.

The obtained data are consistent with literature values.[11]

(±)-2-(1-*t*-Butyldimethylsilyloxy)pent-4-en-2-yloxy)isoindoline-1,3-dione (3b)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79-7.82 (2H, m), 7.70-7.74 (2H, m), 5.93 (1H, m), 5.14 (2H, m), 4.38 (1H, m), 3.90 (1H, m), 3.78 (1H, m), 2.52 (2H, m), 0.76 (9H, s), -0.03 (3H, s), -0.06 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.8, 134.2, 133.2, 129.1, 123.3, 117.9, 8.2, 64.0, 34.6, 25.6, 18.1, -5.7; IR v_{max} 3078, 2922, 1738, 1462, 1188 cm^{-1}; MS (ESI) *m/z* 284 [M+Na]$^+$; HRMS (ESI-TOF) *m/z* [M+Na]$^+$ calculated for $C_{19}H_{27}NO_4SiNa$: 384.1607; found: 384.1608.

(±)-*O*-(1-*t*-Butyldimethylsilyloxy)pent-4-en-2-yloxy)hydroxylamine (4b)

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.82 (1H, m), 5.35 (2H, brs), 5.06 (2H, m), 3.68-3.58 (3H, m), 2.30-2.27 (2H, m), 0.89 (9H, s), 0.05 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 134.9, 116.8, 84.0, 63.7, 34.5, 25.9, 18.3, -5.4; IR v_{max} 3318, 3250, 2954, 2928, 1641, 1256, 837 cm^{-1}; MS (ESI) *m/z* 254 [M+Na]$^+$; HRMS (ESI-TOF) *m/z* [M+H]$^+$ calculated for $C_{11}H_{25}NO_2Si$: 232.1733; found: 232.1733.

(±)-Benzyl 1-(*t*-butyldimethylsilyloxy)pent-4-en-2-yloxycarbamate 5b

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (1H, brs), 7.35-7.37 (5H, m), 5.87 (1H, ddt, J = 10.3, 7.0, 5.9 Hz), 5.16 (2H, s), 5.12 (1H, dd, J = 3.3, 1.6 Hz), 5.08 (1H, dd, J = 3.3, 1.6 Hz), 3.88 (1H, m), 3.73 (2H, m), 2.38-2.33 (2H, ddd, J = 6.2, 2.7, 1.4 Hz), 0.90 (9H, s), 0.07 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.4, 135.7, 134.0, 128.5, 128.3, 128.2, 117.2, 85.3, 67.3, 64.0, 34.2, 25.8, 18.1, -5.5; IR v_{max} 3271, 3069, 2954, 1732, 1454, 1254, 837, 777 cm^{-1}; MS (ESI) *m/z* 388 [M+Na]$^+$; HRMS (ESI-TOF) *m/z* [M+Na]$^+$ calculated for $C_{19}H_{31}NO_4SiNa$: 388.1920; found: 388.1926.

(±)-2-Benzyloxycarbonyl-5-hydroxymethyl-3-methoxyisoxazolidine (6b)

Less polar diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.35 (5H, m), 5.42 (1H, dd, J = 5.9, 1.8 Hz), 5.25 (1H, d, J = 12.2 Hz), 5.19 (1H, d, J = 12.2 Hz), 4.55-4.49 (1H, m), 3.69-3.57 (1H, m) 3.37 (3H, s), 3.29-3.20 (1H, m,), 2.55 (1H, brs), 2.34-2.20 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.7, 135.6, 135.4, 128.7, 128.4, 91.3, 81.5, 68.7, 61.9, 55.9, 36.3.

More polar diastereomer:

^{1}H NMR (400 MHz, $CDCl_3$): δ: 7.38-7.35 (5H, m), 5.46-5.41 (1H, m), 5.24 (1H, d, J = 12.4 Hz), 5.20 (1H, d, J = 12.4 Hz), 4.23 (1H, m), 3.67 (1H, m), 3.87 (1H, m), 3.40 (3H, s), 2.47 (1H, m), 2.23 (1H, ddd, J = 14.5, 7.7, 2.2 Hz), 3.40 (1H, brs), 2.08 (1H, brs); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.0, 135.4, 128.7, 128.6, 128.2, 91.3, 82.1, 68.3, 62.0, 56.0, 36.5; HRMS (ESI-TOF) *m/z* [M+Na]$^+$ calculated for $C_{13}H_{17}NO_5Na$: 290.1004; found: 290.1005; MS (ESI) *m/z* 290 [M+Na]$^+$.

(±)-3-Allyl-2-benzyloxycarbonyl-5-(hydroxymethyl)isoxazolidine (7b)

^{1}H NMR (400 MHz, C_6D_6): δ 7.32-7.38 (5H, m), 5.59 (1H, ddt, *J* = 17.1, 10.4, 6.9 Hz), 4.90 (2H, s), 4.86 (2H, m), 4.04 (1H, m), 3.87 (1H, m), 3.17 (1H, m), 2.93 (2H, ddd, *J* = 12.8, 7.6, 4.9 Hz), 2.16 (1H, m), 1.89 (1H, m), 1.38 (1H, m), 1.29 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.0, 135.6, 134.1, 128.7, 128.8, 128.7, 128.5, 118.2, 81.7, 68.5, 62.1, 59.6, 39.4, 34.3; MS (ESI) *m/z* 300 [M+Na]$^+$; HRMS (ESI-TOF) *m/z* [M+H]+ calculated for $C_{15}H_{19}NO_4$: 278.1392; found: 278.1387.

(±)-Benzyl 3-allyl-5-chloromethyl(isoxazolidine-2-carboxylate (11): Thionyl chloride (1.30 μL, 0.0177 mmol) was added dropwise to a solution of alcohol (**7b**) (9.80 mg, 0.00353 mmol) in anhydrous dichloromethane (5 mL) containing pyridine (1.14 μL, 0.0141 mmol) at 0 °C. The mixture was warmed to room temperature and stirred overnight. Dichloromethane was added followed by ice water. The mixture was extracted with dichloromethane (3 × 10 mL). The combined organic extracts were washed with sodium bicarbonate, dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with (10 % EtOAc / Hexane) to give isoxazolidine (**7a**) (6.10 mg, 58 %) as a colorless clear oil, identical to the material prepared earlier.

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Notes and references

Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, Singapore 637371.

E-mail: roderick@ntu.edu.sg

† Electronic Supplementary Information (ESI) available: Copies of ^{1}H and ^{13}C NMR spectra and computational details. See DOI: 10.1039/b000000x/

1 (a) S. Kondo, S. Shibahara, S. Takahashi, K. Maeda, H. Umezawa and M. Ohno, *J. Am. Chem. Soc.* **1971**, *93*, 6305; (b) M. Hamada, T. Takeuchi, S. Kondo, Y. Ikeda, H. Naganawa, K. Maeda, Y. Okami and H. Umezawa, *J. Antibiot.* **1970,** *23*, 170.

2 (a) S. Shibahara, S. Kondo, K. Maeda, H. Umezawa and M. Ohno, *J. Am. Chem. Soc.* **1972**, *94*, 4353; (b) A. Pierdet, L. Nédélec, V. Delaroff and A. Allais, *Tetrahedron* **1980**, *36*, 1763; (c) G. Pasquet, D. Boucherot and W. R. Pilgrim, *Tetrahedron Lett.* **1980**, *21*, 931; (d) Y.-F. Wang, T. Izawa, S. Kobayashi and M. Ohno, *J. Am. Chem. Soc.* **1982**, *104*, 6465; (e) H. Iida, K. Kasahara and C. Kibayashi, *J. Am. Chem. Soc.* **1986**, *108*, 4647; (f) D. Tanner and P. Somfai, *Tetrahedron Lett.* **1988**, *29*, 2373; (g) S. De Bernardo, J. P. Tengi, G. Sasso and M. Weigele, *Tetrahedron Lett.* **1988**, *29*, 4077; (h) H. Iida, K. Kasahara and C. Kibayashi, *J. Org. Chem.* **1989**, *54*, 2225; (i) U. Schmidt, F. Stabler and A. Lieberknecht *Synthesis* **1992**, 482; (j) C. D. Maycock, M. T. Barros, A. G. Santos, and L. S. Godinho,

Tetrahedron Lett. **1992**, *33*, 4633; (k) J. J. Masters and L. S. Hegedus, *J. Org. Chem.* **1993**, *58*, 4547; (l) D. Socha, J. Jurczak and M. Chmielewski, *Tetrahedron Lett.* **1995**, *36*, 135; (m) S. G. Davies and O. Ichihara, *Tetrahedron*, *Asym.* **1996**, *7*, 1919; (n) J. Jang, Y. Lee and Y. Ahn, *Bull. Kor. Chem. Soc.* **1997**, *18*, 254; (o) M. Shimizu, A. Morita and T. Fujisawa, *Chem. Lett.*, **1998**, 467; (p) R. P. Jain and R. M. Williams, *J. Org. Chem.* **2002**, *67*, 6361; (q) S. V. Naidu and P. Kumar, *Tetrahedron Lett.* **2007**, *48*, 3793; (r) Y. Hayashi, T. Regnier, S. Nishiguchi, M. O. Sydnes, D. Hashimoto, J. Hasegawa, T. Katoh, T. Kajimoto, M. Shiozuka, R. Matsuda, M. Node and Y. Kiso, *Chem. Commun.* **2008**, 2379; (s) S. Nishiguchi, M. O. Sydnes, A. Taguchi, T. Regnier, T. Kajimoto, M. Node, Y. Yamazaki, F. Yakushiji, Y. Kiso and Y. Hayashi, *Tetrahedron* **2010**, *66*, 314; (t) S. G. Davies, O. Ichihara, P. M. Roberts and J. E. Thomson, *Tetrahedron* **2011**, *67*, 216.

3 (a) R. W. Bates and K. Sa-Ei, *Org. Lett.* **2002**, *4*, 4225; (b) R. W. Bates and J. Boonsombat, *Org. Biomol. Chem.*, **2005**, *3*, 520; (c) R. W. Bates, J. A. Nemeth and R. H. Snell, *Synthesis* **2008**, 1033; (d) R. W. Bates, R.H. Snell and S.A. Winbush, *Synlett* **2008**, 1042; (e) R. W. Bates, J. Boonsombat, Y. Lu, J. A. Nemeth, K. Sa-Ei, P. Song, M. P. Cai, P. B. Cranwell and S. A. Winbush *Pure & Applied Chemistry* **2008,** *80*, 681; (f) R. W. Bates and Y. Lu, *J. Org. Chem.* **2009**, *74*, 9460; (g) R. W. Bates and C. J. Lim, *Synlett* **2010**, 866; (h) R. W. Bates and Y. Lu, *Org. Lett.* **2010**, *12*, 3938.

4 (a) R. W. Bates, C. H. Tang, Y. Tan. and S. N. bte Buang, *Synlett* **2012**, 2266; (b) for an application of this reaction, see R. W. Bates, N. F. bte Mohamed Aslam, C. H. Tang and O. Simon, *Tetrahedron*, **2014**, in press.

5 A. D. C. Schuda, P. H. Mazzochi and G. M., T. Fritz, *Synthesis* **1986**, 309. Kumar (ref. 2q) also used this starting material.

6 E. Grochowski and J. Jurczak, *Synthesis* **1976**, 682.

7 DFT calculations were performed at the B3LYP/6-31G* level of theory using Gaussian 09 (Rev. B.01, Gaussian, Inc., Wallingford, CT, 2010). See the Supporting Information for details.

8 Carreira has recently revived a proposal that chlorine substituents can act as neighbouring groups in S_N2 substitution reactions: C. Nilewski, R. W. Geisser and E. M. Carreira, *Nature* **2009**, *457*, 573.

9 P. H. J. Carlsen, T. Katsuki, V. S. Martin and K. B. Sharpless, *J. Org. Chem.* **1981**, *46*, 3936.

10 T. M. V. D. Pinho e Melo, Synthesis of Azides. In *Organic Azides*, John Wiley & Sons, Ltd: 2010; pp 53-94.

11 G. W. Coates, D. Y. Joh and J. W. Kramer, *Org. Lett.* **2007**, *9*, 5581.