Stereoselective Synthesis of Sperabillins and Related Compounds

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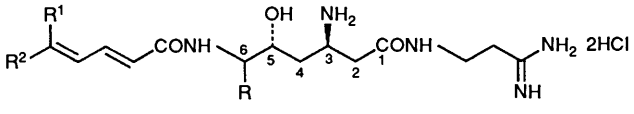
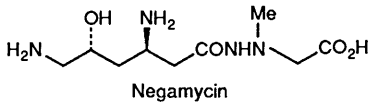
Baker's yeast reduction of methyl (4*S*)-4-(*tert*-butoxycarbonylamino)-3-oxopentanoate **6** stereoselectively afforded methyl (3*R*,4*S*)-*erythro*-4-Boc-amino-3-hydroxypentanoate **7**, which was converted into the *erythro* keto δ-lactone **3a** in three steps. The *threo* keto δ-lactones **3b–c** were diastereoselectively prepared by cyclocondensation of *N*-Boc D- and L-alaninal **4** with 1-methoxy-1,3-bis(trimethylsiloxy)buta-1,3-diene **9** in the presence of a catalytic amount of tin(II) chloride. Reductive amination of the keto lactones **3** using 5% platinum on carbon as catalyst in an acidic medium stereoselectively afforded the *N*-protected 3,6-diamino-5-hydroxyheptanoic acid lactones **1** with 3,5-*anti* stereochemistry. These were transformed into the enantiomerically pure sperabillin **17** and negamycin **20** derivatives in good yields. The configuration of sperabillin B and D was determined to be (3*R*,5*R*,6*R*) by comparison of the synthetic amino lactone **1e** with a degradation product of sperabillin B and by the successful transformation of the synthetic amino lactone **1b** into sperabillin D.

A screening programme for new antibiotics produced by bacteria led to the discovery of a new type of antibiotic, sperabillin (TAN-749) A–D[1] from culture filtrates of *Pseudomonas fluorescens* YK-437. Spectroscopic analyses and degradation studies revealed that sperabillin A and C are unique amino acids, namely (3*R*,5*R*)-3,6-diamino-5-hydroxyhexanoic acids with a 2-amidinoethylamino moiety at the C-1 carboxy group and a (*Z*,*E*)- or (*E*,*E*)-sorbyl moiety at the C-6 amino group. Sperabillin B and D are the 6-methyl congeners of sperabillin A and C, respectively, although the absolute configuration remains unknown.[1] The *in vitro* and *in vivo* antibacterial activities of sperabillin B are slightly more potent than those of sperabillin A,[1] which suggests that the configurationally undefined 6-methyl substituent may have an important effect on the biological activities. Sperabillins have a unique antibacterial profile in that *in vivo* activities are more potent than those expected from the *in vitro* minimum inhibitory concentration values against Gram positive and some Gram negative bacteria.[1] It is also noteworthy that (3*R*,5*R*)-3,6-diamino-5-hydroxyhexanoic acid found in sperabillin A and C is the same constituent as that found in the antibiotic, negamycin.[2] These findings stimulated studies on



Sperabillin A–D

A; R^1 = Me, R^2 = R = H
B; R^1 = R = Me, R^2 = H
C; R^1 = R = H, R^2 = Me
D; R^1 = H, R^2 = R = Me



Negamycin

chemical modification of sperabillins, including studies of structure–activity relations between sperabillin and negamycin derivatives, by both semi- and total-synthetic approaches[3] in the hope of obtaining derivatives with improved antibacterial activities.

Because (3*R*,5*R*)-3,6-diamino-5-hydroxyhexanoic acid is the main constituent of negamycin, it and its related compounds have become attractive targets for enantioselective syntheses, and a number of excellent syntheses starting from chiral pools[4] or enzymatically-derived chiral building blocks,[5] as well as by asymmetric synthesis[6] have been published. However, these processes appear to be inapplicable to the general synthesis of sperabillin derivatives, in which two amino groups have to be distinguishable and introduction of a substituent at the C-6 position is desired in a stereochemically fixed manner.

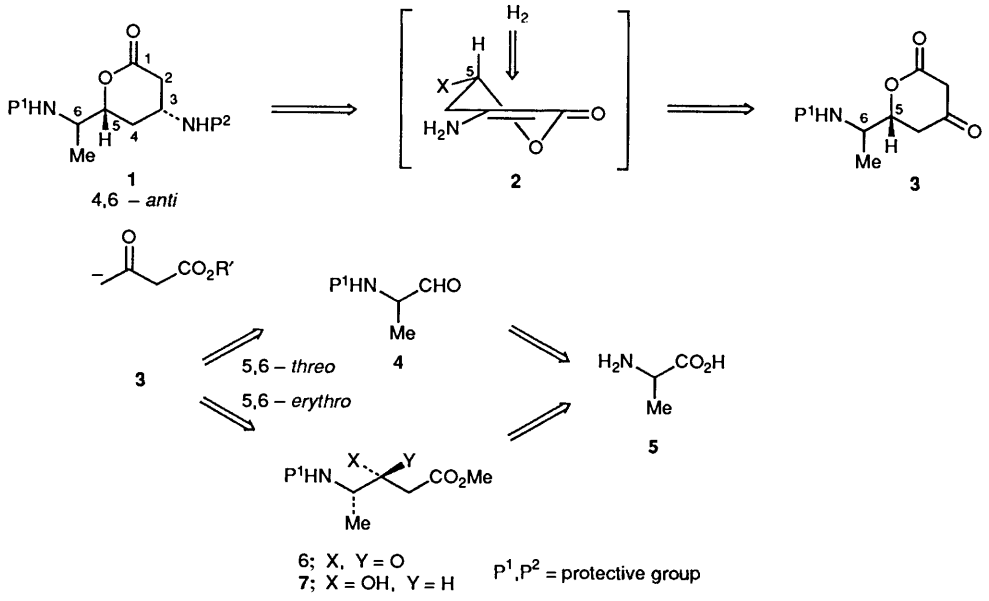
In this paper we describe an enantioselective synthesis of the *N*-protected (3*R*,5*R*,6*R*)-3,6-diamino-5-hydroxyheptanoic acid lactone and its stereoisomers **1** *via* stereoselective reductive amination of the optically active keto δ-lactone **3a–c**, and an efficient transformation of **1a–c** into sperabillin and negamycin derivatives. Determination of the configuration at C-6 position of sperabillin B and D is also described.

Results and Discussion

We planned a synthetic route to sperabillins using the amino lactones **1**, which had been isolated from the degradation studies[1] of sperabillins, as the key intermediates. Retro-synthetic analysis of **1** is shown in Scheme 1, in which the key feature is the stereoselective reduction of the enamine **2** derived from the optically active keto lactone **3**; the desired 3,5-*anti* relative stereoechemistry† would be formed by preferential approach of the reducing agent from the upper side of the chair-like conformation of the enamine **2**, which could be controlled stereoelectronically.[7] The optically active *threo* and *erythro* keto lactones **3**, on the other hand, would be prepared stereoselectively starting from alanine **5** *via* **4** and **6**, respectively, as shown in Scheme 1.

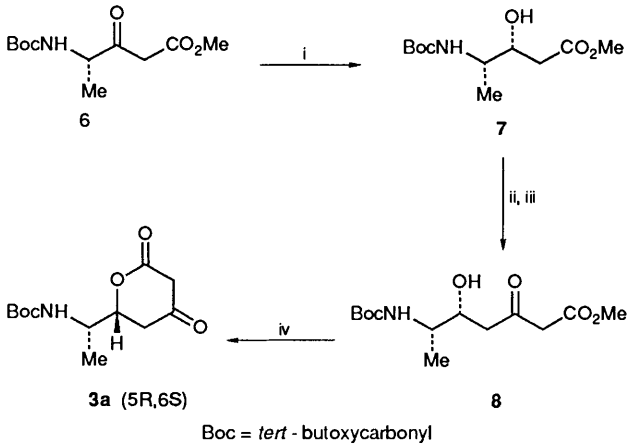
Synthesis of the Keto Lactones **3** *from Amino Acids.*—Chemical reduction of methyl (4*S*)-4-(*tert*-butoxycarbonylamino)-3-oxopentanoate **6**, prepared from *N*-Boc (*tert*-butoxycarbonyl) L-alanine by the reported procedure,[8] using borohydrides in various conditions gave the *erythro* isomer **7** as the

† For clarity, numbering and suffixes (*syn*, *anti*) of the linear form are used for the lactone form throughout the paper other than for names given in the Experimental section (see Figures and Schemes).



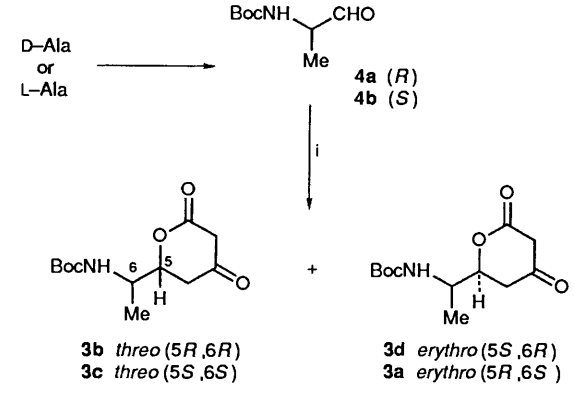
Scheme 1

major product.* However, the stereoselectivity was not satisfactory for practical use, ranging from 4:1 to 1:1 estimated by ^{1}H NMR spectroscopic analyses. The observed stereoselectivity is similar to that recently reported in the reduction of a similar type of β-keto esters with chemical reducing agents.[10] On the other hand, baker's yeast reduction[11] of the keto ester **6** was found to achieve high *erythro* diastereoselectivity [97:3 (ratio of the crude product), 86% chemical yield]. Simple recrystallization of the crude product from isopropyl ether increased the diastereoisomeric excess to 98.4%. Hydrolysis of the resulting *erythro* ester **7** followed by successive treatment with *N*,*N*'-carbonyldiimidazole (CDI) and the magnesium salt of hydrogen methyl malonate gave the keto ester **8** (57.0%), which was transformed into the *erythro* keto lactone **3a** by treatment with aqueous sodium hydroxide (99.9%) (Scheme 2).



Scheme 2 *Reagents and conditions:* i, Baker's yeast; ii, aq. NaOH; iii, CDI, $Mg(O_2CCH_2CO_2Me)_2$–THF; iv, aq. NaOH

In order to obtain the *threo* keto lactone **3c**, condensation of *N*-Boc L-alaninal **4b** with some γ-anion synthons of 3-oxobutanoic acid derivatives was investigated. Among them, cyclocondensation[12] of **4b** with 1-methoxy-1,3-bis(trimethylsiloxy)buta-1,3-diene **9**[13] (3 equiv.) (Scheme 3) in the presence of a catalytic amount of tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyloctane-3,5-dionato)europium(III) [Eu(fod)$_3$] was revealed to afford the keto lactone **3c** + **3a** in the ratio of 7:1 (determined by HPLC analysis) in moderate yield (~61%). Comparison of ^{1}H NMR spectroscopic data of the product with those of the *erythro* keto lactone **3a** revealed that the major isomer is the desired *threo* keto lactone **3c**; the 6-Me signals are diagnostic for the stereochemical assignment [δ 1.26 (doublet) for **3a**, and δ 1.36 (doublet) for **3c**].

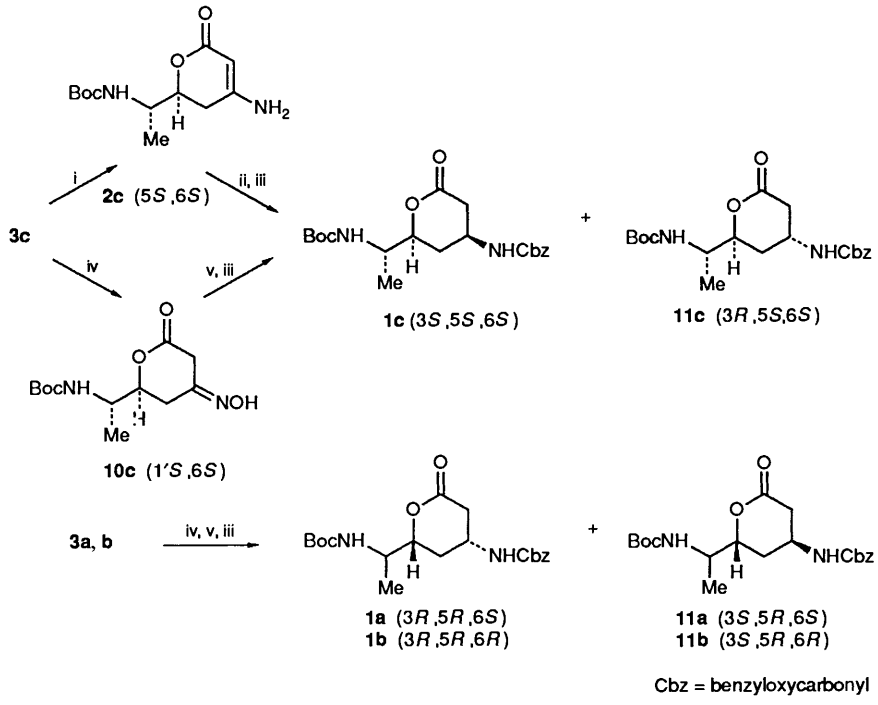


Scheme 3 *Reagents and conditions:* i, CH_2=C(OSiMe$_3$)CH=C(OSiMe$_3$)(OMe) **9**, Lewis acid catalyst–CH_2Cl_2

Optimal conditions for the cyclocondensation were explored using *N*-Boc D-alaninal **4a** and 2 equiv. of the diene **9** (Table 1). Boron trifluoride–diethyl ether, Eu(fod)$_3$ and tin(II) salts showed similar diastereoselectivity in the reaction, and among them tin(II) triflate achieved the highest diastereoselectivity, although the isolated yield was low (entry 4). The reaction condition employing tin(II) chloride as catalyst at 5–8 °C was the optimal condition tested from the point of view of a balance between diastereoselectivity and chemical yield (entry 3). The observed *threo* selectivity in these reactions could be rationalized by the chelation control model.[12b,e–g,14]

Stereoselective Reductive Amination of the Keto Lactones **3**.—Treatment of the *threo* keto lactone **3c** with an excess of

* The relative stereochemistry was determined by the oxazolidinone method used for determination of the stereochemistry of statine and its analogues.[9]



Scheme 4 *Reagents and conditions:* i, NH_4OAc–$CHCl_3$, reflux; ii, $NaBH_3CN$ or H_2, H^+, catalyst; iii, CbzCl, aq. $NaHCO_3$–THF; iv, NH_2OH–HCl, pyridine–MeOH; v, H_2, H_3PO_4–P_2O_5, 5% Pt/C

Table 1 Effect of catalyst on the cyclocondensation reaction of **4a** with **9**[a]

Entry	Catalyst (mol %)	Yield (%)	Ratio of the isomers[b] **3b** : **3d**
1	BF_3OEt_2 (2)	21.1	85 : 15
2	$Eu(fod)_3$ (1)	39.0	88 : 12
3	$SnCl_2$ (2)	41.3	95 : 5
4	$Sn(OTf)_2$ (2)	6.1	99.5 : 0.5
5	SnF_2 (2)	26.2	91 : 9
6	$SnSO_4$ (2)	39.7	87 : 13

[a] 1-Methoxy-1,3-bis-(trimethylsiloxy)buta-1,3-diene **9** (2 equiv.) in CH_2Cl_2 at 5–8 °C for 16 h. [b] The ratio was estimated by HPLC of the crude products.

ammonium acetate afforded the enamine **2c** in 72% yield under azeotropic conditions, by refluxing in chloroform in the presence of molecular sieves (Scheme 4). Our first attempt at the reduction of the enamine **2c** with cyanoborohydrides in acidic media did not give the desired diastereoselectivity* (Table 2, entry 1). The desired *anti* diastereoselectivity was observed in the catalytic hydrogenation of the enamine **2c** using platinum dioxide in the presence of phosphoric acid to give a 3:1 diastereoisomeric mixture of the *anti* isomer **1c** and the *syn* isomer **11c** (entry 2). The more manageable catalyst, 5% platinum on carbon was also effective for the reduction (entry 3). On the other hand, the oxime **10c**, which was easily prepared in almost quantitative yield from the keto lactone **3c** by treatment with hydroxylamine, proved to be practically more useful than the enamine **2c** as a substrate for the catalytic hydrogenation; monitoring of the reaction by TLC demonstrated that the oxime **10c** was rapidly converted into the enamine **2c**, which was further reduced to give a diastereoisomeric mixture with the same ratio as that obtained by reduction of the enamine itself (entry 4). In this reaction, however, a substantial amount of the keto lactone **3c** was recovered, which was presumed to be formed by hydrolysis of the intermediate enamine **2c** with water that was generated as a result of reduction of the oxime. To suppress the side reaction, addition of phosphorus pentoxide as a dehydrating agent was examined. This proved to be effective at improving the overall yield; the desired *anti* isomer **1c** was isolated from the crude product by preferential crystallization in 33% overall yield from **3c** (entry 5). Reductive amination of the enantiomer **3b** and the diastereoisomer **3a** *via* the oximes gave similar results, forming the corresponding *anti* isomer as the major product (entry 6, 7). These results suggest that alteration of the C-6 configuration has little effect on the diastereoselectivity in the reduction. The stereoselective formation of the desired *anti* isomer in the above reactions is consistent with the mechanistic consideration described in the retro-synthetic analysis.

Reductive amination of the linear hydroxy keto ester **8** was examined for comparison. Reduction of **8** with sodium cyanoborohydride in the presence of ammonium acetate followed by *tert*-butoxycarbonylation gave a diastereoisomeric mixture of the amino alcohols **12a–b** in *ca.* 10:1 ratio, from which the major isomer **12a** was isolated as crystals in 23% yield† after purification by column chromatography. Hydrolysis of the major isomer **12a** followed by lactonization using 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (WSC·HCl) afforded the amino lactone **11d**, whose stereochemistry was revealed to be *syn* (3*S*,5*R*,6*S*) by ^1H NMR spectroscopic analysis as described below. The *syn* selectivity

* The ratio of the diastereoisomers described in this section is that estimated by ^1H NMR spectroscopic (200 MHz) analyses of the unseparated isomers obtained after benzyloxycarbonylation of the crude products [the integration of the $C_{2\text{-eq}}$ proton signal in the *anti* isomers **1** and $C_{2\text{-ax}}$ proton signal in the *syn* isomers **11** were used for this estimation (see Experimental section)]: the stereochemical assignment is described in the main text (*vide infra*).

† The low yield is due to the concomitant formation of diols in the reduction.

Table 2 Conditions for the reductive amination of the keto lactones **3** *via* **2** or **10**

Entry	Substrate	Conditions	Yield[a] (%)	Ratio of isomer[b] **1** (*anti*) : **11** (*syn*)	
1	**2c**	$NaBH_3CN$, AcOH–THF	50	1	1
2	**2c**	H_2,[c] cat. PtO_2, H_3PO_4–THF	41	3	1
3	**2c**	H_2, cat. 5% Pt–C, H_3PO_4–THF	72 (38)[d]	3	1
4	**10c**	H_2, cat. 5% Pt–C, H_3PO_4–THF	60	3	1
5	**3c**[e]	H_2, cat. 5% Pt–C, H_3PO_4–P_2O_5–THF	85[e] (33)[d]	3	1
6	**3b**[e]	H_2, cat. 5% Pt–C, H_3PO_4–P_2O_5–THF	86[e] (33)[d]	3	1
7	**3a**[e]	H_2, cat. 5% Pt–C, H_3PO_4–P_2O_5–THF	85[e] (40)[d]	3	1

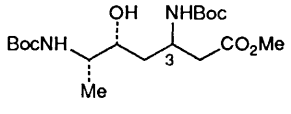
[a] Crude yield after benzyloxycarbonylation. [b] The ratio was estimated by ^1H NMR (200 MHz) spectroscopic anslyses of the unseparated isomers obtained after benzyloxycarbonylation of the crude reduction products; the integration of the $C_{2\text{-eq}}$ proton signal in the *anti* isomers **1** and the $C_{2\text{-ax}}$ proton signal in the *syn* isomers **11** were used for this estimation (see Experimental section). [c] Carried out at 1 atom. in entry 2–7. [d] In parentheses, isolated yield of the major (*anti*) isomer by crystallization from $(Pr^i)_2O$. [e] *via* the oxime, and the overall yield from **3** is represented.

Table 3 ^1H NMR chemical shifts (δ) of the *anti* and *syn* lactones **1d–e** and **11d**[a]

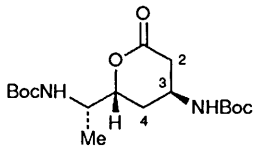
	2-H		$J_{2,3}$ (Hz)			4-H		
	axial	equatorial	$J_{2\text{-ax},3}$	$J_{2\text{-eq},3}$	J_{gem} (Hz)	axial	equatorial	Me
1d (*anti-erythro*)	2.35, (Δ0.63)	2.98	9.3,	6.6	17.6	1.40–1.60, (Δ0.9)	2.21–2.34	1.18
1e (*anti-threo*)	2.33, (Δ0.64)	2.97	9.3,	6.4	17.5	1.50–1.61, (Δ0.8)	2.19–2.28	1.28
11d (*syn-erythro*)	2.77, (Δ −0.14)	2.63	6.1,	4.3	17.6	1.85, (Δ0.15)	2.00	1.19

[a] In $CDCl_3$ (400 MHz).

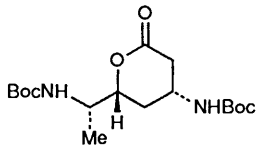
in the reductive amination of the hydroxy keto ester **8** may be explained by a transition state similar to that proposed for the reduction of β-hydroxy oximes with lithium aluminium hydride, which was reported to afford the *syn* amino alcohols stereoselectively.[15]



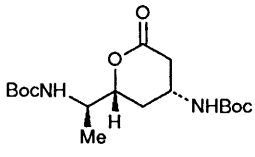
12a (3*S*)
12b (3*R*)



11d (3*S*,5*R*,6*S*)



1d (3*R*,5*R*,6*S*)



1e (3*R*,5*R*,6*R*)

Assignment of *syn* and *anti* stereochemistry in the amino lactones **1** and **11** is based on NMR spectroscopic evidence. The diagnostic signals in ^1H NMR spectra of *N*,*N*′-di-Boc-amino derivatives of (3*R*,5*R*,6*S*) **1d**,* (3*R*,5*R*,6*R*)-*anti-threo* **1e**,* and (3*S*,5*R*,6*S*) **11d** lactones are shown in Table 3. The differences in chemical shifts between the geminal protons at the C-2 and C-4 positions are larger for the *anti* isomers **1d–e** (Δ0.63–0.64 and Δ0.8–0.9) than for the *syn* isomer **11d** (Δ −0.14, and Δ0.15), and the coupling constant ($J_{2\text{-ax},3}$) in the *anti* isomers **1d–e** (9.3 Hz) is larger than that in the *syn* isomer **11d** (6.1 Hz); these data are in good correlation with those reported for the similar systems.[16]

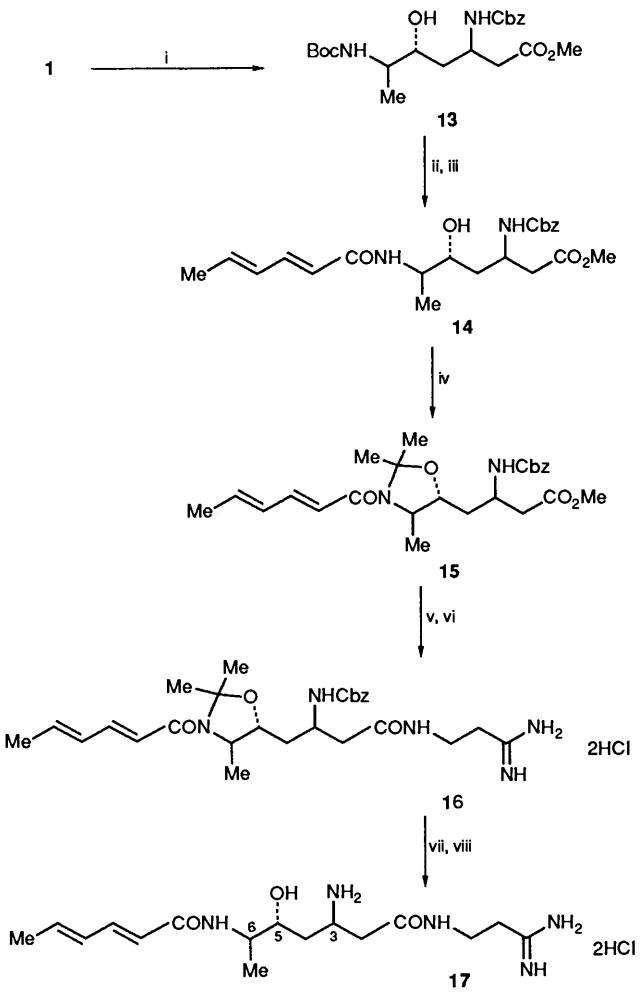
The synthetic amino lactone **1e** was identical with the *N*,*N*′-di-Boc amino lactone derived from natural sperabillin B,[17] indicating that the configuration of the C-6 position of sperbillin B and D is *R*.

Synthesis of Sperabillin and Negamycin Derivatives from the Amino Lactones **1**.—Transformation of the *anti* amino lactones **1a–c** into sperabillin C and D, and related compounds **17a–c** was achieved by the procedure shown in Scheme 5. Treatment of the amino lactones **1a–c** with methanol in the presence of a catalytic amount of base afforded the methyl esters **13a–c** in good yields. Deprotection of the Boc group by hydrogen chloride in methanol and subsequent acylation with (*E*,*E*)-sorbyl chloride gave the esters **14a–c**. The secondary alcohols which might engage in formation of lactones in the next step were protected as acetonides **15a–c**. Hydrolysis of the ester group of **15a–c** and subsequent condensation with 2-amino-ethylamidine dihydrochloride[18] using dicyclohexylcarbodi-imide (DCC) and 1-hydroxybenzotriazole (HOBT) afforded the protected sperabillin derivatives **16a–c**, which were converted into the sperabillin derivatives **17a–c** by an iodotrimethylsilane mediated deprotection reaction.[19] The spectroscopic data of synthetic sperabillin D **17b** were identical with those of the naturally occurring compound.

The negamycin derivatives **20a–c** possessing a methyl substituent at the C-6 position were also prepared from the methyl esters **13a–c** by the method shown in Scheme 6. The acetonides **18a–c** obtained from **13a–c** were converted into the protected negamycin derivatives **19a–c** by hydrolysis and subsequent condensation with benzyl *N*-methylhydrazino-acetate toluene-*p*-sulphonate[20] using DCC and HOBT. The stepwise deprotection of **19a–c** afforded the negamycin derivatives **20a–c** in good yields.

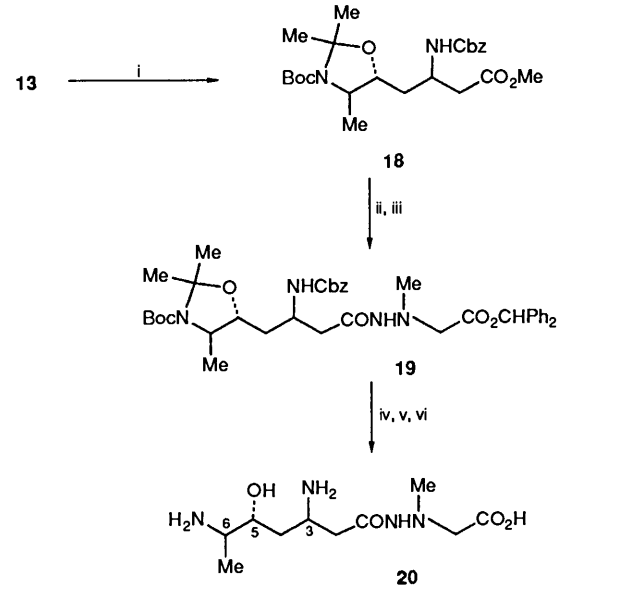
In vitro antibacterial activity of the synthetic sperabillin D **16b** was compatible with that of natural sperabillin D. To our

* The compounds **1d–e** were isolated from the corresponding keto lactones **3a** and **3b** *via* the oximes by the reductive amination as described above followed by treatment with di(*tert*-butyl) dicarbonate.



1, 13–17 [a; (3*R*,5*R*,6*S*), b; (3*R*,5*R*,6*R*), c; (3*S*,5*S*,6*S*)]

Scheme 5 *Reagents and conditions:* i, cat. $Pr^i_2NEt_2$ or NaOMe/MeOH; ii, HCl–MeOH; iii, MeCH=CHCH=CHCOCl, pyridine; iv, 2-methoxypropene, cat. *p*-TsOH–Py; v, aq. NaOH; vi, HOBT, DCC, 2-aminoethylamidine hydrochloride; vii, Me_3SiI–MeCN; viii, IRA-401 (Cl^-)



13, 18–20 [a; (3*R*,5*R*,6*S*), b; (3*R*,5*R*,6*R*), c; (3*S*,5*S*,6*S*)]

Scheme 6 *Reagents and conditions:* i, 2-methoxypropene, cat. *p*-TsOH–Py; ii, aq. NaOH; iii, HOBT, DCC, *p*-TsOH $H_2NN(Me)CH_2CO_2CHPh_2$, Et_3N; iv, H_2, 10% Pd/C; v, CF_3CO_2H; vi, CG-50W (NH_4^+)

regret *in vitro* antibacterial activities of 6-*epi*-sperabillin D **16a** and the enantiomer of sperabillin D **16c** were substantially lower. The C-6 methyl derivatives of negamycins **20a–c** showed reduced *in vitro* antibacterial activities compared with negamycin; the structure–activity relationship is in contrast to that observed in sperabillin A–D.

Application of this synthetic process to the synthesis of sperabillin and negamycin derivatives having various other substituents at the C-6 position is now ongoing.

Experimental

M.p.s were determined using a Yanagimoto m.p. apparatus and are uncorrected. IR spectra were measured with a Hitachi 215 spectrophotometer, and mass spectra with JEOLTMS-DX303 and Hitachi M-80A mass spectrometers. ^1H NMR spectra were taken on a Varian EM-390 (90 MHz), a Varian Gemini 200 (200 MHz) and a JNM-GX400FT (400 MHz) spectrometer with tetramethylsilane or sodium {2,2,3,3-[$^2H_4$]-3-(trimethylsilyl)-proprionate} as internal standard, *J* values are given in Hz. Optical rotations α were recorded with a JASCO DPI-181 digital polarimeter and are given in 10^{-1} deg dm^2 g^{-1}. HPLC data were obtained using a Hitachi 655A equipment, and the analytical conditions were as follows: Ultrasphere-ODS; pH 3.5 buffer solution–acetonitrile (v/v). Extracted solutions were dried over anhydrous magnesium sulphate.

Methyl (4S)-4-(tert-*Butoxycarbonylamino*)-3-*oxopentanoate* **6**.—*N*,*N*′-Carbonyldiimidazole (CDI) (19.4 g, 0.12 mol) was added portionwise to a solution of *N*-*tert*-butoxycarbonyl (Boc)-L-alanine (18.9 g, 0.1 mol) in dry THF (200 cm^3) at 5 °C and the mixture was stirred for 1 h at room temperature. In a separate flask a suspension of magnesium chloride (9.2 g, 96.6 mmol) and the potassium salt of hydrogen methyl malonate (23.4 g, 0.15 mol) in dry THF (150 cm^3) was stirred for 4 h at 50 °C. To the suspension, the aforementioned imidazolide solution was added at room temperature. The mixture was stirred for 14 h at room temperature, concentrated under reduced pressure, and the concentrate suspended in ethyl acetate. The suspension was washed successively with aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate and brine, dried and evaporated to dryness. Chromatography of the residue on silica gel with hexane–ethyl acetate as eluent (3:1–1:1, v/v) gave the *keto ester* **6** (16.6 g, 67.8%) as colourless crystals. Recrystallization from isopropyl ether–hexane provided an analytical sample, m.p. 57–58 °C (Found: C, 53.8; H, 7.8; N, 5.3; $C_{11}H_{19}NO_5$ requires C, 53.9; H, 7.8; N, 5.7%); δ_H(90 MHz; $CDCl_3$) 1.35 (3 H, d, *J* 7, Me), 1.43 (9 H, s, Me), 3.55 (3 H, s, OMe), 4.35 (1 H, m, CH) and 5.15 (1 H, br, NH); $[\alpha]_D^{26}$ −53.7 (*c* 0.99, MeOH).

Methyl (3R,4S)-4-(tert-*Butoxycarbonylamino*)-3-*hydroxypentanoate* **7**.—A mixture of saccharose (4.0 g), dry yeast (2.0 g, Oriental Yeast Co., Ltd. Dry Yeast) and water (50 cm^3) was stirred for 1h at room temperature. The keto ester **6** (0.49 g, 2.00 mmol) was rinsed into the mixture with ethanol (1 cm^3) and the resulting mixture was stirred for 5 d at room temperature. The reaction mixture was filtered and the cake was washed with ethyl acetate. The combined filtrate and washings were extracted with ethyl acetate. The extract was washed with brine and then dried. The solution was evaporated to dryness and the residue was chromatographed on silica gel. Gradient elution with hexane–ethyl acetate (1:0–3:2, v/v) gave the *hydroxy ester* **7** (0.43 g, 87.0%) as colourless crystals. Recrystallization of the crude product from isopropyl ether gave an analytical sample, m.p. 92–93 °C (Found: C, 53.3; H, 8.5; N, 5.6. $C_{11}H_{21}NO_5$ requires C, 53.4; H, 8.6; N, 5.7%); δ_H(400 MHz; $CDCl_3$) 1.14 (3 H, d, *J* 7,

Me), 1.43 (9 H, s, Me), 2.46 (2 H, d, *J* 6, CH_2), 3.30 (1 H, d, *J* 4, OH), 3.70 (3 H, s, OMe), 3.65–3.74 (1 H, m, CH), 3.95–4.04 (1 H, m, CH) and 4.68–4.72 (1 H, br, NH); $[\alpha]_D^{26}$ −9.7 (*c* 1.06, MeOH).

Methyl (5R,6S)-6-(tert-*Butoxycarbonylamino*)-5-*hydroxy*-3-*oxoheptanoate* **8**.—Aqueous sodium hydroxide (5 mol dm^{-3}; 9.4 cm^3, 47.16 mmol) was added to a solution of **7** (10.6 g, 42.87 mmol) in a mixture of dioxane (60 cm^3), methanol (20 cm^3) and water (30 cm^3) at 5 °C. The mixture was stirred for 6 h at room temperature and then concentrated under reduced pressure. The concentrate was dissolved in water and washed with ether. The aqueous layer was acidified with aqueous potassium hydrogen sulphate and extracted with ethyl acetate. The extract was dried and concentrated under reduced pressure, to give (3R,4S)-4-(tert-*butoxycarbonylamino*)-3-*hydroxypentanoic acid* (9.61 g, 96.1%) as colourless crystals, m.p. 105–106 °C (from ethyl acetate–hexane) (Found: C, 51.5; H, 8.15; N, 6.0. $C_{10}H_{19}NO_5$ requires C, 51.5; H, 8.2; N, 6.0%); ν_{max}(Nujol)/cm^{-1} 3360, 1710, 1685, 1325, 1170 and 1040; δ_H(90 MHz); [$^2H_6$]DMSO) 1.00 (3 H, d, *J* 6, Me), 1.37 (9 H, m, Bu^t), 1.90–2.50 (2 H, m, CH_2), 3.11–3.92 (4 H, m, 2 × CH and 2 × OH) and 6.43–6.56 (1 H, br, NH); $[\alpha]_D^{26}$ −9.8 (*c* 1.02, MeOH). The pentanoic acid (9.50 g, 41.0 mmol) thus obtained was dissolved in dry THF (250 cm^3) and to this solution was added dropwise at 5 °C over 3 h *N*,*N*-carbonyldiimidazole (CDI) (7.21 g, 47.16 mmol) in dry THF (100 cm^3) followed by the magnesium methyl malonate (7.21 g, 27.86 mmol). The reaction mixture was stirred for 18 h at room temperature and then concentrated under reduced presure. The concentrate was dissolved in ethyl acetate and the solution was washed with water, aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate and brine, dried, and concentrated under reduced pressure. The concentrate was chromatographed on silica gel. Gradient elution with hexane–ethyl acetate (1:2–0:1) gave the *keto ester* **8** (6.6 g, 59.3%) as colourless crystals, m.p. 85–86 °C (from Et_2O) (Found: C, 54.05; H, 8.0; N, 4.85. $C_{13}H_{23}NO_6$ requires C, 54.0; H, 8.0; N, 4.8%); ν_{max}(Nujol)/cm^{-1} 3450, 1750, 1710 and 1680; δ_H(90 MHz; $CDCl_3$) 1.13 (3 H, d, *J* 7, Me), 1.43 (9 H, s, Bu^t), 2.67 (2 H, d, *J* 6, CH_2CO), 3.50 (2 H, s, CH_2), 3.73 (3 H, s, OMe) 4.05 (1 H, m, CH) and 4.76 (1 H, d, *J* 8, NH); $[\alpha]_D^{26}$ −5.9 (*c* 1.035, MeOH).

(6R)-6-[(1S)-1-(tert-*Butoxycarbonylamino*)*ethyl*]-5,6-*dihydro*-2H-*pyran*-2,4(3H)-*dione* **3a**.—Aqueous sodium hydroxide (1 mol dm^{-3}; 18.5 cm^3) was added dropwise to a solution of **8** (4.85 g, 16.76 mmol) in THF (50 cm^3) at 5 °C. The mixture was stirred for 1.5 h at room temperature and then evaporated. The residue was dissolved in water and the solution was washed with ethyl acetate. The aqueous layer was acidified with aqueous potassium hydrogen sulphate and extracted with ethyl acetate. The extract was dried and concentrated under reduced pressure. Treatment of the concentrate with isopropyl ether gave the *keto lactone* **3a** (4.32 g, 99.9%) as colourless crystals, m.p. 144–145 °C (from ethyl acetate–hexane) (Found: C, 56.2; H, 7.5; N, 5.4. $C_{12}H_{19}NO_5$ requires C, 56.0; H, 7.4; N, 5.4%); ν_{max}(Nujol)/cm^{-1} 3380, 1750, 1735, 1720 and 1685; δ_H(90 MHz; $CDCl_3$) 1.26 (3 H, d, *J* 7, Me), 1.43 (9 H, s, Bu^t), 2.64 (2 H, d, *J* 3, CH_2), 3.50 (2 H, d, *J* 3, CH_2), 3.75–3.83 (1 H, m, CH) and 4.73–4.79 (1 H, m, CH); $[\alpha]_D^{23}$ +2.4 (*c* 0.63, MeOH).

General Procedure for Cycloaddition of N-*Protected* α-*Amino Aldehydes* **4** *with the Siloxy Diene* **9**.—Lewis acid catalyst was added to a anhydrous methylene dichloride solution of *N*-protected α-amino aldehydes **4** (0.5 mol dm^{-3}), which were prepared from α-amino acids by the reported method,[21] at −30 °C under argon and then the siloxy diene **9**[13] (2–3 equiv.) was added to the mixture in one portion. The reaction mixture was stirred at 5–8 °C for 12–16 h and then quenched by the addition of aqueous potassium hydrogen sulphate. The organic layer was extracted with aqueous sodium carbonate. The combined extracts were washed with methylene dichloride, acidified with potassium hydrogen sulphate, and then extracted with ethyl acetate. The extract was washed with brine, dried and evaporated to give a diastereoisomeric mixture of the products. Diastereoisomeric ratios were determined by HPLC. The results are summarized in Table 1. The physicochemical data of **3b** and **3c**, which were isolated as colourless crystals by recrystallization [from chloroform–carbon tetrachloride (2:1)] of the crude products, are as follows:

(6S)-6-[(1S)-1-(tert-*Butoxycarbonylamino*)*ethyl*]-5,6-*dihydro*-2H-*pyran*-2,4(3H)-*dione* **3c**. When 3 equiv. of the diene **9** was used, the crude *product* (**3c**:**3a**, 7:1) was obtained in 61.0% yield. For 2 equiv. of diene **9**, the pure **3c** was obtained in 40.9% yield after recrystallization, m.p. 147–149 °C (Found: C, 55.7; H, 7.4; N, 5.3. $C_{12}H_{19}NO_5$ requires C, 56.0; H, 7.4; N, 5.4%); ν_{max}(KBr)/cm^{-1} 3370, 2980, 1680, 1620, 1585, 1515 and 1290; δ_H(90 MHz; $CDCl_3$) 1.36 (3 H, d, *J* 7, Me), 1.43 (9 H, s, Bu^t), 2.60 (1 H, d, *J* 4, 5-H), 2.70 (1 H, s, 5-H), 3.50 (2 H, ABq, 3-H), 3.80–4.24 (1 H, m, 1′-H) and 4.50–4.94 (2 H, m, 6-H and NH); $[\alpha]_D^{23}$ −114.1 (*c* 0.44, $CHCl_3$).

(6R)-6-[(1R)-1-(tert-*Butoxycarbonylamino*)*ethyl*]-5,6-*dihydro*-2H-*pyran*-2,4(3H)-*dione* **3b**. Yield 43.1%, m.p. 146–148 °C (Found: C, 55.9; H, 7.5; N, 5.4. $C_{12}H_{19}NO_5$ requires C, 56.0; H, 7.4; N, 5.4%); $[\alpha]_D^{23}$ +117.6 (*c* 0.25, $CHCl_3$); the IR and ^1H NMR spectra were identical with those of **3c**.

(6S)-4-*Amino*-6-[(1S)-1-(tert-*butoxycarbonylamino*)*ethyl*]-5,6-*dihydro*-2H-*pyran*-2-*one* **2c**.—A mixture of the keto lactone **3c** (500 mg, 1.943 mmol), ammonium acetate (1.0 g), molecular sieves 4 Å (5.0 g) and chloroform (20 cm^3) was refluxed for 3 h, and then filtered. The filtrate was concentrated under reduced pressure and the concentrate was diluted with ethyl acetate. The solution was washed with water, aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate, and brine, dried, and concentrated under reduced pressure. Treatment of the concentrate with chloroform–hexane gave the *enamine* **2c** (360 mg, 72.2%) as colourless crystals, m.p. 172–174 °C (Found: C, 56.0; H, 7.9; N, 10.7. $C_{12}H_{20}N_2O_4$ requires C, 56.2; H, 7.9; N, 10.9%); ν_{max}(KBr)/cm^{-1} 3350, 3160, 1690, 1640, 1575, 1520, 1245 and 1165; δ_H(90 MHz; $CDCl_3$) 1.34 (3 H, d, *J* 8, Me), 1.45 (9 H, s, Bu^t), 2.12 (1 H, dd, *J* 4 and 18, 5-H), 2.68 (1 H, dd, *J* 13 and 18, 5-H), 3.63–4.08 (1 H, m, 1′-H), 4.20–4.42 (1 H, m, 6-H), 4.79–4.98 (1 H, m, NH), 4.89 (1 H, s, =CH) and 5.06–5.25 (2 H, br, NH_2); $[\alpha]_D^{23}$ −135.0 (*c* 0.18, $CHCl_3$).

General Procedure for Preparation of the Oximes **10**.—Pyridine (2–3 equiv.) was added dropwise to a suspension of hydroxylamine hydrochloride (1.3:1.5 equiv.) and the β-keto lactones **3** in methanol (0.5 mol dm^{-3}) at 5 °C. The mixture was stirred for 1 h at 5 °C and then after 3 h at room temperature was evaporated. The residue was dissolved in ethyl acetate and the solution was washed with water, aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate and brine, dried, and concentrated to give the crude oximes **10**. These were used in the subsequent reaction without purification and characterization except for **10c**.

(6S)-6-[(1S)-1-(tert-*Butoxycarbonylamino*)*ethyl*]-5,6-*dihydro*-2H-*pyran*-2,4(3H)-*dione* 4-*Oxime* **10c**. The crude product was obtained as pale yellow *crystals* which appeared to be a 1:1 mixture of (*E*)- and (*Z*)-isomers by ^1H NMR spectroscopic analysis; yield 99.5%, m.p. 195–198 °C (decomp.) (from ether–ethyl acetate) (Found: C,52.9; H, 7.4; N, 10.1. $C_{12}H_{20}N_2O_5$ requires C, 52.9; H, 7.4; N, 10.3%); ν_{max}(KBr)/cm^{-1} 3360, 2980, 1735, 1680, 1530, 1165, 1045 and 970; δ_H(200 MHz; $CDCl_3$ +

[$^2H_6$]DMSO) 1.30 × 2 (3 H, d × 2, *J* 7, CH_3), 1.44 and 1.45 (9 H, s × 2, Bu^t), 2.43–2.99 (2 H, m, 5-H), 3.41 (1 H, s, 3-H), 3.52 (1/2 H, dd, *J* 1 and 21.5, 3-H), 3.69 (1/2 H, d, *J* 21.5, 3-H), 3.90–4.20 (1 H, m, 1′-H), 4.28–4.51 (1 H, m, 6-H), 4.80–4.94 (1 H, m, NH) and 10.23–10.26 (1 H, br, OH).

General Procedure for Reductive Amination of the Oximes **10** *or the Enamine* **2c** *with Platinum–Carbon Catalyst and Following Benzyloxycarbonylation or* tert-*Butoxycarbonylation.*—5% Platinum–carbon (*ca.* equal to double weight of the substrate), phosphoric acid (20% weight of the catalyst), and phosphorus pentoxide (20% weight of the catalyst) were added to a solution of the oximes **10** or the enamine **2c** in dry THF (0.1–0.3 mol dm^{-3}) at room temperature. The mixture was vigorously stirred under hydrogen. The reaction was monitored by TLC (8–24 h). The catalyst was filtered off and washed with water–THF (1:1). The combined filtrate and washings were adjusted at pH 8 with sodium hydrogen carbonate and then benzyloxycarbonyl chloride (Cbz–Cl) (1.5–2 equiv.) or di-*tert*-butyl dicarbonate (1.5–2 equiv.) was added to the mixture at 5 °C. The mixture was stirred for 3 h at room temperature and then twice extracted with ethyl acetate. The combined extracts were dried and concentrated under reduced pressure and the concentrate was purified by column chromatography on silica gel to give a mixture of diastereoisomers of the amino lactones, from which the major isomers (*anti*) **1a–e** were isolated by crystallization from isopropyl ether [the minor isomers (*syn*) **11** were not isolated in pure forms]. The results are summarized in Table 2. The physicochemical data of the major isomers **1a–e** are as follows:

(4R,6R)-4-*Benzyloxycarbonylamino*-6-[(1S)-1-(tert-*butoxycarbonylamino*)*ethyl*]*tetrahydropyran*-2-*one* **1a**. Colourless *crystals*; yield 40.3% from **3a** *via* the oxime, m.p. 133–134 °C (Found: C, 61.4; H, 7.3; N, 7.05. $C_{20}H_{28}N_2O_6$ requires C, 61.2; H, 7.2; N, 7.1%); ν_{max}(KBr)/cm^{-1} 3350, 2900, 1750, 1690, 1520, 1470, 1360, 1280, 1240, 1160, 1040 and 730; δ_H(200 MHz; $CDCl_3$) 1.18 (3 H, d, *J* 6.8, Me), 1.44 (9 H, s, Bu^t), 1.50–1.68 (1 H, m, 5-H), 2.20–2.34 (1 H, m, 5-H), 2.37 (1 H, dd, *J* 9.2 and 17.6, 3-H), 3.01 (1 H, ddd, *J* 1.4, 6.5 and 17.6, C_{3-eq}-H), 3.73–3.92 (1 H, m, CH), 3.98–4.20 (1 H, m, CH), 4.22–4.39 (1 H, m, CH), 4.50–4.92 (2 H, m, NH), 5.11 (2 H, s, OCH_2) and 7.36 (5 H, s, Ar); $[\alpha]_D^{22}$ −34.0 (*c* 0.65, EtOH). The diastereoisomer ratio of the reaction product was estimated by the ^1H NMR (200 MHz) spectroscopic analyses using 3-H_{ax}, which appears at δ 2.81 (dd, *J* 6.2 and 17.7) in the *syn* isomer **11a**, and 3-H_{eq} in the *anti* isomer **1a**.

(4R,6R)-4-*Benzyloxycarbonylamino*-6-[(1R)-1-(tert-*butoxycarbonylamino*)*ethyl*]*tetrahydropyran*-2-*one* **1b**. Colourless *crystals*; yield 32.8% from **3b** *via* the oxime, m.p. 121–123 °C (Found: C, 61.0; H, 7.2; N, 7.0. $C_{20}H_{28}N_2O_6$ requires C, 61.2; H, 7.2; N, 7.1%); $[\alpha]_D^{26}$ +18.3 (*c* 0.23, $CHCl_3$). The IR and ^1H NMR spectra were identical with those of **1c**.

(4S,6S)-4-*Benzyloxycarbonylamino*-6-[(1S)-1-(tert-*butoxycarbonylamino*)*ethyl*]*tetrahydropyran*-2-*one* **1c**. Colourless *crystals*; yield 23.4% from **2c**, and yield 33.2% from **3c** *via* the oxime **10c**, m.p. 123–124 °C (Found: C, 61.1; H, 7.2; N, 7.1. $C_{20}H_{28}N_2O_6$ requires C, 61.2; H, 7.2; N, 7.1%); ν_{max}(KBr)/cm^{-1} 3230, 2980, 1700br, 1530, 1300, 1275 and 1240; δ_H(200 MHz; $CDCl_3$) 1.23 (3 H, d, *J* 7, Me), 1.43 (9 H, s, Bu^t), 1.50–1.80 (1 H, m, 5-H), 2.02–2.36 (1 H, 5-H), 2.32 (1 H, dd, *J* 9 and 18, 3-H), 2.95 (1 H, dd, *J* 6 and 18, 3-H_{eq}), 3.70–4.30 (3 H, m, CHN and CHO), 4.62 (1 H, d, *J* 10, NH), 5.02 (1 H, d, *J* 10, NH), 5.10 (2 H, s, CH_2Ph) and 7.35 (5 H, s, Ar); $[\alpha]_D^{23}$ −17.0 (*c* 0.235, $CHCl_3$). The diastereoisomer ratio of the reaction product was estimated by the ^1H NMR (200 MHz) spectroscopic analyses using 3-H_{ax}, which appears at δ 2.79 (dd, *J* 6.0 and 17.4) in the *syn* isomer **11c**, and 3-H_{eq} in the *anti* isomer **1c**.

(4R,6R)-4-tert-*Butoxycarbonylamino*-6-[(1S)-1-(tert-*butoxycarbonylamino*)*ethyl*]*tetrahydropyran*-2-*one* **1d**. Colourless *crystals*; yield 35.0% from **3a** *via* the oxime, m.p. 163–164.5 °C (Found: C, 56.9; H, 8.45; N, 7.7 requires C, 57.0; H, 8.4; N, 7.8%); ν_{max}(KBr)/cm^{-1} 3375, 2990, 1720, 1680, 1520, 1450, 1390, 1360, 1240 and 1160; δ_H(400 MHz; $CDCl_3$) 1.18 (3 H, d, *J* 6.8, Me), 1.44 × 2 (9 H × 2, 2 × s, Bu^t), 1.40–1.60 (1 H, m, 5-H), 2.21–2.34 (1 H, m, 5-H), 2.35 (1 H, dd, *J* 9.3 and 17.6, 3-H), 2.98 (1 H, ddd, *J* 1.2, 6.6 and 17.6, 3-H), 3.77–3.88 (1 H, m, 1′-H), 3.96–4.14 (1 H, m, 4-H), 4.30 (1 H, d, *J* 11.5, 6-H), 4.64 (1 H, d, *J* 7.8, NH) and 4.81–4.95 (1 H, br, NH); $[\alpha]_D^{22.5}$ −32.4 (*c* 0.52, $CHCl_3$).

(4R,6R)-4-tert-*Butoxycarbonylamino*-6-[(1R)-1-(tert-*butoxycarbonylamino*)*ethyl*]*tetrahydropyran*-2-*one* **1e**. Colourless *crystals*; yield 36.8% from **3b** *via* the oxime, m.p. 165–165.5 °C (Found: C, 57.1; H, 8.5; N, 7.1 requires C, 57.0; H, 8.4; N, 7.8; ν_{max}(KBr)/cm^{-1} 3360, 2980, 1715, 1690, 1530, 1365, 1235, 1165 and 1045; δ_H(400 MHz; $CDCl_3$) 1.28 (3 H, d, *J* 7.1, Me), 1.48 (18 H, s, Bu^t), 1.50–1.61 (1 H, m, 5-H), 2.19–2.28 (1 H, m, 5-H), 2.33 (1 H, dd, *J* 9.3 and 17.5, 3-H), 2.97 (1 H, ddd, *J* 1.5, 6.4 and 17.5, 3-H), 3.83–3.95 (1 H, m, 1′-H), 3.99–4.15 (1 H, m, 4-H), 4.24 (1 H, d, *J* 11.6, 5-OH), 4.44–4.60 (1 H, br, NH) are 4.62 (1 H, d, *J* 8.6, NH); $[\alpha]_D^{22.5}$ +21.1 (*c* 0.46, $CHCl_3$); these data were identical with those of the di-Boc amino lactone from sperabillin B.[17]

Methyl (3S,5R,6S)-3,6-*Bis*(tert-*Butoxycarbonylamino*)-5-*hydroxyheptanoate* **12a**.—To a stirred mixture of compound **8** (289 mg, 1 mmol), ammonium acetate (620 mg, 8 mmol) and molecular sieves 3 Å (0.5 g) in MeOH (15 cm^3) was added portionwise sodium cyanoborohydride (0.30 g, 4.7 mmol). The mixture was stirred for 3 h at room temperature and then filtered. The filtrate was evaporated and to the residue was added water (6 cm^3) and ethyl acetate (15 cm^3). The mixture was acidified at pH 3 with aqueous potassium hydrogen sulphate with ice cooling, and then made basic with aqueous potassium carbonate. To this mixture was added di(*tert*-butyl dicarbonate (0.4 cm^3). After the mixture had been stirred at room temperature for 3 h, the organic layer was separated, washed with water, dried and evaporated. The residue was chromatographed on silica gel using hexane–ethyl acetate (2:1, v/v) as eluent. The first fractions gave a mixture of the (3*R*)-isomer of the title compound **12b** and unidentified compound as a colourless oil (16 mg). Comparison of the ^1H NMR spectrum of the mixture with that of the (3*R*,5*R*,6*S*)-isomer **12b**, which was prepared from **1d** (*vide infra*), showed that the content of **12b** in the mixture is *ca.* 50%. The second fractions gave the *amino alcohol* **12a** (91 mg, 23.4%) as colourless crystals (recrystallized from isopropyl ether), m.p. 126–127 °C (Found: C, 55.5; H, 8.5; N, 7.25. $C_{18}H_{34}N_2O_7$ requires C, 55.4; H, 8.8; N, 7.2%); *m*/*z* 391 (M + 1) and 390 (M^+); ν_{max}(KBr)/cm^{-1} 3370, 2980, 1710, 1690, 1520, 1365, 1245, 1165 and 1050; δ_H(200 MHz; $CDCl_3$) 1.11 (3 H, d, *J* 6.5, Me), 1.44 (18 H, s, Bu^t), 1.58–1.77 (2 H, m, 4-H), 2.56 (1 H, dd, *J* 5.4 and 15.9, 2-H), 2.68 (dd, *J* 5.6 and 15.9, 2-H), 3.70 (3 H, s, OMe), 3.58–3.84 (2 H, m, CH), 3.95–4.19 (1 H, m, CH), 4.72–4.87 (1 H, br, NH) and 5.28–5.45 (1 H, br, NH); $[\alpha]_D^{23}$ −12.7 (*c* 0.26, MeOH).

(4S,6R)-4-tert-*Butoxycarbonylamino*-6-[(1S)-1-(tert-*butoxycarbonylamino*)*ethyl*]*tetrahydropyran*-2-*one* **11d**.—A mixture of compound **12a** (60 mg, 0.15 mmol), NaOH (1 mol dm^{-3}; 0.2 cm^3) and THF (2 cm^3) was stirred for 2.5 h at room temperature and then evaporated. Water was added to the residue and the mixture was acidified with aqueous potassium hydrogen sulphate and then extracted with ethyl acetate. The extract was washed with water, dried and evaporated to give the acid of **11a** as colourless crystals, which was, without purification,

dissolved in chloroform (5 cm^3). To the solution was added WSC·HCl (80 mg). After being stirred for 0.5 h at room temperature, the mixture was diluted with chloroform, washed with water, dried and evaporated to give the lactone **11d** (48 mg, 89.0%) as colourless crystals, m.p. 143–145 °C (Found: C, 57.15; H, 8.5; N, 7.6. $C_{17}H_{30}N_2O_6$ requires C, 57.0; H, 8.4; N, 7.8%); δ_H(400 MHz; $CDCl_3$) 1.19 (3 H, d, *J* 6.8, Me), 1.44 (9 H, s, Bu^t), 1.45 (9 H, s, Bu^t), 1.85 (1 H, ddd, *J* 4.9, 11.8 and 14.2, 5-H), 2.00 (1 H, d, *J* 14.2, 5-H), 2.63 (1 H, dd, *J* 4.3 and 17.6, 3-H), 2.77 (1 H, dd, *J* 6.1 and 17.6, 3-H), 3.76–3.88 (1 H, m, 1′-H), 4.08–4.20 (1 H, m, 4-H), 4.55 (1 H, d, *J* 11.5, 6-H), 4.70–4.96 (1 H, br, NH) and 4.92 (1 H, d, *J* 8.1, NH); $[\alpha]_D^{22.5}$ −40.6 (*c* 0.49, $CHCl_3$).

Methyl (3R,5R,6S)-3,6-*Bis*(tert-*Butoxycarbonylamino*)-5-*hydroxyheptanoate* **12b**.—According to the general procedure for methanolysis of the amino lactone **1** as described below, the amino lactone **1d** (6 mg) was subjected to methanolysis using diisopropylethylamine as the base to give the methyl ester **12b** (6 mg), as a colourless oil; *m/z* 391 (M + 1) and 390 (M^+); δ_H(200 MHz; $CDCl_3$) 1.10 (3 H, d, *J* 6.6, Me), 1.44 (18 H, s, Bu^t), 1.40–1.66 (2 H, m, 4-H), 2.51 (1 H, m, dd, *J* 5.4 and 16.1, 2-H), 2.66 (1 H, dd, *J* 4.8 and 16.1, 2-H), 3.52–3.76 (1 H, m, CH), 3.70 (3 H, s, OMe), 4.02–4.25 (2 H, m, CH), 4.78–5.04 (1 H, br, NH) and 5.43 (1 H, d, *J* 8.0, NH).

General Procedure for Methanolysis of the Amino Lactones **1**.—A solution (0.05 cm^3) containing a base [methanol (2 cm^3) solution of diisopropylethylamine (0.02 cm^3), or methanol (2 cm^3) solution of sodium methoxide (10 mg)] was added to a solution of the amino lactones **1** in methanol (0.1–0.2 mol dm^{-3}) at room temperature. The mixture was stirred for 16 h at room temperature and then evaporated. The residue was dissolved in ethyl acetate and the solution washed with water, aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate and brine, dried and concentrated to give the methyl esters **13** which were used in the subsequent reaction without further purification.

General Procedure for the Deprotection of BOC Group in the Methyl Esters **13** *and Following Acylation with* (E,E)-*Sorbyl Chloride*.—Gaseous hydrogen chloride was bubbled into a solution of the ester **13** in methanol (0.2–0.5 mol dm^{-3}) at 5 °C with TLC monitoring of the reaction. After completion of the reaction the mixture was evaporated and the residue was dissolved in methylene dichloride (0.2–5.0 mol dm^{-3}). Pyridine (3–5 equiv.) was added to the solution at 5 °C, and then (*E,E*)-sorbyl chloride (1.5–2 equiv.) was added dropwise to the mixture at 5 °C. The mixture was stirred for 1–2 h at 5 °C and then concentrated under reduced pressure. The concentrate was diluted with ethyl acetate and washed with water, aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate and brine, dried and concentrated under reduced pressure. The concentrate was chromatographed on silica gel to give the products **14**. The physicochemical data are as follows.

Methyl (3R,5R,6S)-3-*benzyloxycarbonylamino*-6-[(E,E)-*hexa*-2,4-*dienoylamino*]-5-*hydroxyheptanoate* **14a**. Colourless *crystals* recrystallized from ether; yield 51.6%, m.p. 148–150 °C; ν_{max}(KBr)/cm^{-1} 3300, 1725, 1695, 1665, 1650, 1605, 1530 and 1260; δ_H(90 MHz; $CDCl_3$) 1.10 (3 H, d, *J* 7, Me), 1.40–1.73 (2 H, m, CH_2), 1.86 (3 H, d, *J* 5, CH_3), 2.58 (2 H, d, *J* 5, CH_2CO), 3.63 (3 H, s, OMe), 3.50–4.45 (3 H, m, CHO and CHN), 5.10 (2 H, s, OCH_2Ph), 5.60–6.21 and 7.00–7.60 (4 H, m, olefinic) and 7.32 (5 H, s, Ar).

Methyl (3R,5R,6R)-3-*benzyloxycarbonylamino*-6-[(E,E)-*hexa*-2,4-*dienoylamino*]-5-*hydroxyheptanoate* **14b**. Colourless *crystals* recrystallized from ether; yield 46.9%, m.p. 136–139 °C (Found: C, 63.35; H, 7.0; N, 7.0. $C_{22}H_{30}N_2O_6$ requires C, 63.1; H, 7.2; N, 6.7%); the IR and ^{1}H NMR spectra were identical with those of **14c**.

Methyl (3S,5S,6S)-3-*benzyloxycarbonylamino*-6-[(E,E)-*hexa*-2,4-*dienoylamino*]-5-*hydroxyheptanoate* **14c**. Colourless *crystals* recrystallized from ether; yield 57.9%, m.p. 136–140 °C; ν_{max}(KBr)/cm^{-1} 3420, 3300, 1725, 1655, 1625, 1605, 1535 and 1255; δ_H(90 MHz; $CDCl_3$) 1.23 (3 H, d, *J* 7, Me), 1.43–1.75 (2 H, m, CH_2), 1.82 (3 H, d, *J* 5, =$CHCH_3$), 2.55 (2 H, t, *J* 5, CH_2CO), 3.37–3.70 (2 H, m, CHN), 3.65 (3 H, s, OMe), 3.85–4.35 (3 H, m, CHO, OH and NH), 5.11 (2 H, s, OCH_2), 5.58–6.20 and 7.10–7.45 (4 H, m, olefinic) and 7.33 (5 H, s, Ar); $[\alpha]_D^{23}$ −10.4 (*c* 0.135, $CHCl_3$).

General Procedure for Protection of the Hydroxy Group with Acetonide.—A catalytic amount (5–10 mol %) of pyridinum-toluene-*p*-sulphonate was added to a solution of the methyl esters **13** or **14** in a 10:1 mixture of methylene dichloride and 2-methoxypropene (0.2–0.5 mol dm^{-3}) at room temperature. The mixture was stirred for 3–24 h at room temperature and then concentrated under reduced pressure. The concentrate was diluted with ethyl acetate and washed with water, aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate and brine, dried, and concentrated under reduced pressure. The concentrate was purified by column chromatography on silica gel to give the acetonides **18** or **15**. The crude products were used in the subsequent reaction without further purification.

General Procedure for Preparation of the Sperabillin Derivatives **17** *from the Acetonides* **15**.—Aqueous sodium hydroxide (2.5 mol dm^{-3}; 3–5 equiv.) was added to a solution of the acetonides **15** in methanol–THF (2:1; 0.2–0.5 mol dm^{-3}) at 5 °C. The mixture was stirred for 3–5 h at room temperature and then evaporated. The concentrate was dissolved in water and washed with ether. The aqueous layer was acidified with aqueous potassium hydrogen sulphate and twice extracted with ethyl acetate. The extract was dried and concentrated under reduced pressure. The concentrate was dissolved in dry THF (0.2–0.5 mol dm^{-3}), and *N*-hydroxybenzotriazole (HOBT) (1.1–1.3 equiv.) and 1,3-dicyclohexylcarbodiimide (DCC) (1.2–1.5 equiv.) were added to the solution at room temperature. The mixture was stirred for 1–3 at room temperature after which the resulting precipitates were filtered off and washed with THF. To the combined filtrate and washings, a solution of 2-aminoethylamidine dihydrochloride (1.0–1.1 equiv.) and sodium hydrogen carbonate (1.5–2.2 equiv.) in water (1–2 mol dm^{-3}) were added at room temperature. The reaction mixture was stirred for 16 h at room temperature, and then concentrated under reduced pressure. The concentrate was dissolved in chloroform–ethanol (3:1) dried, and then concentrated under reduced pressure. To a suspension of the concentrate in dry acetonitrile (0.2–0.5 mol dm^{-3}), trimethylsilyl iodide (3.0–5.0 equiv.) was added at room temperature. The mixture was stirred for 2–4 h at room temperature and concentrated under reduced pressure. The concentrate was dissolved in water and adjusted at pH 3 with dilute hydrochloric acid. The solution was washed with ether and concentrated under reduced pressure. The concentrate was subjected to chromatography on XAD-II and eluted with water. The eluate was subjected to chromatography on IRA-401 (Cl^-) and eluted with water. Lyophilization of the eluate gave the sperabillin derivatives **17** as a hygroscopic pale yellow powder. The physicochemical data of the products are as follows.

3-{(3R,5R,6S)-3-*Amino*-6-[(E,E)-*hexa*-2,4-*dienoylamino*]-5-*hydroxyheptanoyl*}*aminopropanamidine Dihydrochloride* **17a**. Yield 68.7% from **14a**, SIMS *m/z* 340 (M^+); ν_{max}(KBr)/cm^{-1} 3420(br), 1690, 1650 and 1340; δ_H(90 MHz; D_2O) 1.25 (3 H,

d, *J* 7, Me), 1.75–2.20 (5 H, m, Me and CH_2), 2.81 (2 H, d, *J* 7, CH_2CO), 2.68–3.02 [2 H, m, $CH_2C(=NH)NH_2$], 3.65 (2 H, *J* 7, CH_2N), 3.50–4.43 (3 H, m, CHO and CHN), 5.93–6.65 and 7.10–7.45 (4 H, m, olefinic); $[\alpha]_D^{26}$ −33.3 (*c* 0.15, H_2O).

3-[(3R,5R,6R)-3-*Amino*-6-(E,E)-*hexa*-2,4-*dienoylamino*-5-*hydroxyheptanoyl*]*aminopropanamidine Dihydrochloride* **17b**. Yield 68.7% from **13b**, SIMS *m/z* 340 (M + 1), 341 (M + 2); $[\alpha]_D^{26}$ +30.6 (*c* 0.64, H_2O) [lit.,[1] $[\alpha]_D$ +30.4 (*c* 0.50, H_2O)]; the IR, ^1H NMR spectra, and HPLC were identical with those of natural sperabillin D.

3-[(3S,5S,6S)-3-*Amino*-6-(E,E)-*hexa*-2,4-*dienoylamino*-5-*hydroxyheptanoyl*]*aminopropanamidine Dihydrochloride* **17c**. Yield 16.8% from **15c** (Found: C, 41.6; H, 8.0; N, 14.7. $C_{16}H_{29}N_5O_3 \cdot 2HCl \cdot 3H_2O$ requires C, 41.2; H, 8.0; N, 15.0%); SIMS *m/z* 340 (M + 1), 341 (M + 2); ν_{max}(KBr)/cm^{-1} 3260(br), 1685, 1650, 1630 and 1335; δ_H(90 MHz; D_2O) 1.28 (3 H, d, *J* 7, Me), 1.68–2.12 (2 H, m, CH_2), 1.90 (3 H, d, *J* 5, Me), 2.77 (2 H, t, *J* 9, CH_2), 2.80 (2 H, d, *J* 7, CH_2CO), 3.53–4.35 (5 H, m, CHN and CHO), 5.96–6.48 and 7.10–7.40 (4 H, m, olefinic); $[\alpha]_D^{25}$ −29.6 (*c* 0.415, H_2O).

General Procedure for Preparation of the Negamycin Derivatives **20** *from the Acetonides* **18**.—Aqueous sodium hydroxide (2.5 mol dm^{-3}; 3–5 equiv.) was added to a solution of the acetonide derivatives **18** in methanol–THF (2:1; 0.2–0.5 mol dm^{-3}) at 5 °C. The mixture was stirred for 3–5 h at room temperature and then evaporated. The residue was dissolved in water and washed with ether. The aqueous layer was acidified with aqueous potassium hydrogen sulphate and extracted with ethyl acetate. The extract was dried and concentrated under reduced pressure. The concentrate was dissolved in dry THF (0.2–0.5 mol dm^{-3}) and HOBT (1.1–1.3 equiv.) and DCC (1.2–1.5 equiv.) were added to the solution at room temperature. The mixture was stirred for 1–3 h at room temperature. The resulting precipitates were filtered off and washed with THF. To the combined filtrate and washings, benzyl methylhydrazinoacetate (1.1 equiv.)[20] was added at room temperature. The mixture was stirred for 16 h at room temperature and diluted with ethyl acetate. The mixture was washed with water, aqueous sodium hydrogen carbonate and brine, dried (Na_2SO_4) and concentrated under reduced pressure. The concentrate was dissolved in methanol–5% acetic acid (2:1; 0.2–0.5 mol dm^{-3}) and 10% palladium–carbon (the same weight as the substrate) was added to the solution. The suspension was stirred at room temperature under hydrogen for 2 h after which the catalyst was filtered off and washed with methanol. The combined filtrate and washings were concentrated under reduced pressure and the concentrate was dissolved in trifluoroacetic acid (0.3 mol dm^{-3}). The solution was stirred for 2 h at room temperature and concentrated under reduced pressure. The concentrate was subjected to chromatography on CG-50W(NH_3^+) or Dowex-50W(H^+) and eluted with diluted aqueous ammonia. Lyophilization of the eluate gave the negamycin derivatives **20** as a colourless hygroscopic powder. The physicochemical data of the products are as follows:

2-(3R,5R,6S)-3,6-*Diamino*-5-*hydroxyheptanoyl*-1-*methylhydrazinoacetic acid* **20a**. Yield 91.4% from **13a**, SIMS *m/z* 263 (M^+); ν_{max}(KBr)/cm^{-1} 3700–2400br, 1650, 1580, 1400 and 1310; δ_H(90 MHz; D_2O) 1.30 (3 H, d, *J* 7, Me), 1.55–1.98 (2 H, m, CH_2), 2.51 (2 H, d, *J* 7, CH_2CO), 2.72 (3 H, s, NMe), 3.36–3.66 (4 H, m, NH_2 and CH) and 4.07–4.13 (1 H, m, CH); $[\alpha]_D^{25}$ +10.0 (*c* 0.10, H_2O).

2-(3R,5R,6R)-3,6-*Diamino*-5-*hydroxyheptanoyl*-1-*methylhydrazinoacetic acid* **20b**. Yield 64.1% from **13b** (Found: C, 42.4; H, 8.4; N, 19.8. $C_{10}H_{22}N_4O_4 \cdot 1.2H_2O$ requires C, 42.3; H, 8.7; N, 19.7%); ν_{max}(KBr)/cm^{-1} 3400–2900br, 1655, 1580, 1400 and 1315; δ_H(90 MHz; D_2O) 1.33 (3 H, d, *J* 7, Me), 1.60–1.95 (2 H, m, CH_2), 2.47 (2 H, d, *J* 7, CH_2CO), 2.72 (3 H, s, NMe), 2.95–4.00 (3 H, m, CHN and CHO) and 3.49 (2 H, s, NCH_2CO); $[\alpha]_D^{22}$ +14.0 (*c* 0.25, H_2O).

2-(3S,5S,6S)-3,6-*Diamino*-5-*hydroxyheptanoyl*-1-*methylhydrazinoacetic Acid* **20c**. Yield 51.8% from **13c** (Found: C, 42.0; H, 8.5; N, 19.2. $C_{10}H_{22}N_4O_4 \cdot 1.4H_2O$ requires C, 41.8; H, 8.7; N, 19.5%); δ_H(90 MHz; D_2O) 1.35 (3 H, d, *J* 7, Me), 1.60–2.05 (2 H, m, CH_2), 2.46 (2 H, d, *J* 7, CH_2CO), 2.71 (3 H, s, NMe), 2.85–4.00 (3 H, m, CHN and CHO) and 3.48 (2 H, s, NCH_2CO); $[\alpha]_D^{22}$ −14.6 (*c* 0.185, H_2O).

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