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Synthesis and Activity of Analogues of the Isoleucyl tRNA Synthetase Inhibitor SB-203207

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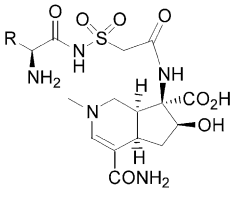
Abstract—Twenty two analogues of SB-203207 have been prepared by total synthesis, and evaluated as inhibitors of a range of tRNA synthetases. Changes to the bicyclic core, removing either the terminal amino substituent or the sulfonyl group from the side chain, and altering either the carbon skeleton or stereochemistry of the isoleucine residue, decreases the potency of inhibition of isoleucyl tRNA synthetase. Substituting the isoleucine residue with other amino acids produces inhibitors of the corresponding synthetases. In particular, a methionine derivative is 50–100 times more potent against methionyl tRNA synthetase than against any of the corresponding isoleucyl, leucyl, valyl, alanyl and prolyl synthetases.

Introduction

In connection with a high-throughput screening program aimed to identify new anti-infective agents, SB-203207 (**1a**) was isolated from *Streptomyces* NCIMB 40513 and shown to inhibit isoleucyl tRNA synthetase (IRS) from *Staphylococcus aureus* Oxford and rat liver, with IC_{50} values of 1.7 and <2 nM, respectively.[1,2] Since the yield of the inhibitor **1a** from natural sources was insufficient to allow for further testing and the preparation of analogues, we obtained altemicidin (**2**) through fermentation and used it to prepare a semi-synthetic sample of **1a** and several other compounds with the same bicyclic core.[3] In parallel studies, we undertook the total synthesis of a more structurally diverse but related series of compounds, in order to establish comprehensive structure–activity relationships. Accordingly, we recently reported the multi-component assembly of the bicyclic alcohols (±)-**3**-(±)-**5** and the elaboration of (±)-**3** to the diastereomeric analogues **8a(I)** and **8a(II)** of SB-203207 (**1a**).[4] We now report the synthesis of a further 22 analogues of **1a** from (±)-**3**-(±)-**5**, and the evaluation of the activity of these compounds as inhibitors of a range of amino acyl tRNA synthetases.



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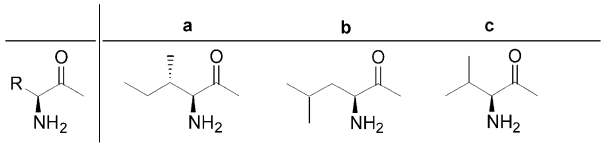

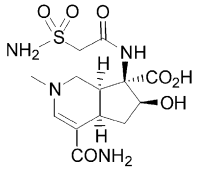

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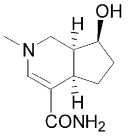
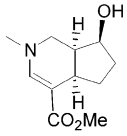
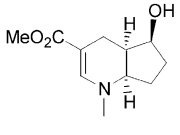


(±)-**3**



(±)-**4**



(±)-**5**

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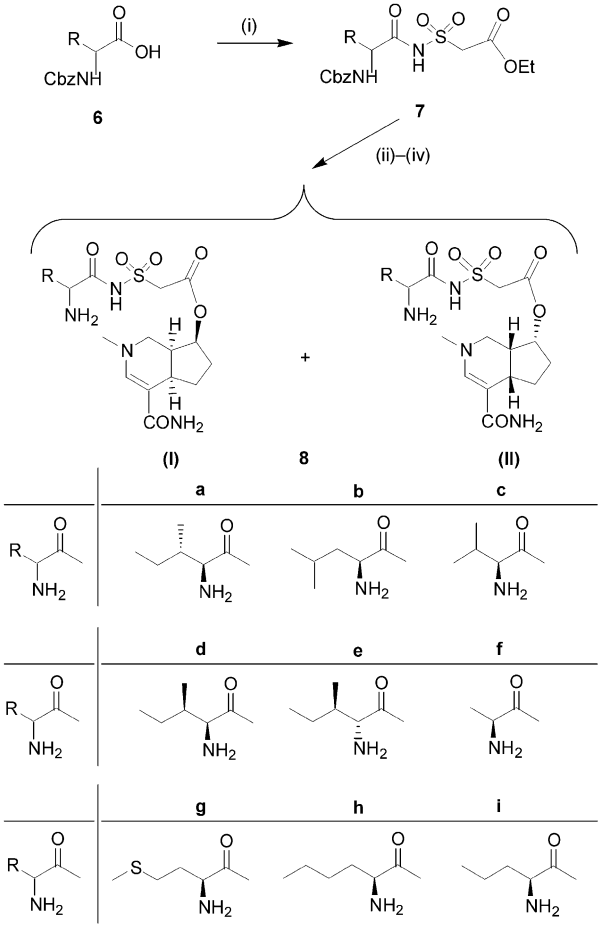
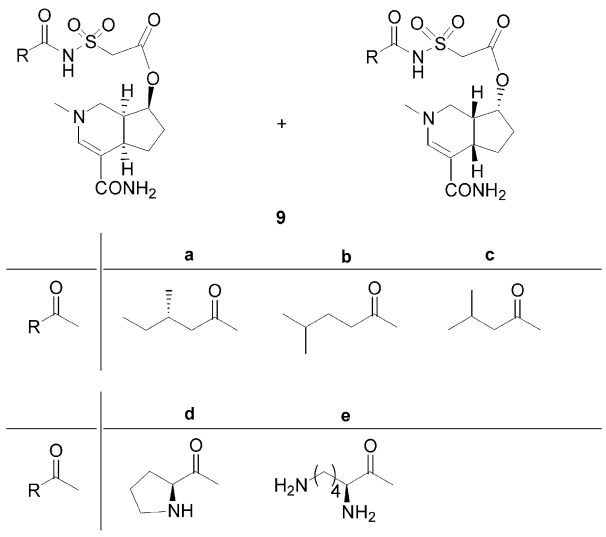
Results and Discussion

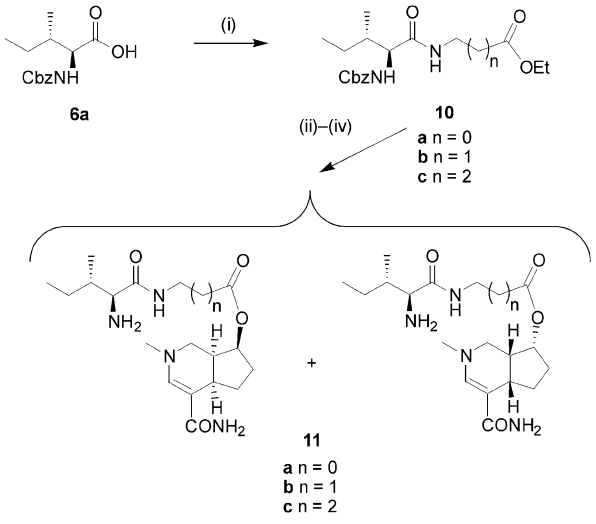
The methods used to elaborate the alcohols (±)-**3**-(±)-**5** are illustrated in Schemes 1–4. As shown in Scheme 1, EDCI-mediated coupling of the protected amino acids **6a–i** with ethyl 2-sulfamylacetate afforded **7a–i**. Base hydrolysis of these esters gave the corresponding carboxylic acids. The acids reacted with oxalyl chloride, and the product acid chlorides were treated with the bicyclic alcohol (±)-**3** in the presence of triethylamine and a catalytic amount of DMAP. Catalytic hydrogenolysis of the products then gave **8a–i**, respectively. Similar methods were used to prepare **9a–e**, **11a–c** (Scheme 2), **12a–c** (Scheme 3) and **13a–c** (Scheme 4), except that the ethyl esters of glycine, β-alanine and γ-aminobutyric acid were used instead of ethyl 2-sulfamylacetate to prepare **11a–c**, and the alcohols (±)-**4** and (±)-**5** were used instead of (±)-**3** to prepare **12a–c** and **13a–c**, respectively. Compounds **8a–i**, **9a–e**, **11a–c**, **12a–c** and **13a–c** were each obtained as a ca. 1/1 mixture of diastereomers, as illustrated. They were isolated as either the hydrochloride or acetate salts, by freeze-drying from hydrochloric acid or acetic acid solutions, respectively. Samples of the individual diastereomers **8a(I)** and **8a(II)** were also prepared using the separated enantiomers of **3**.



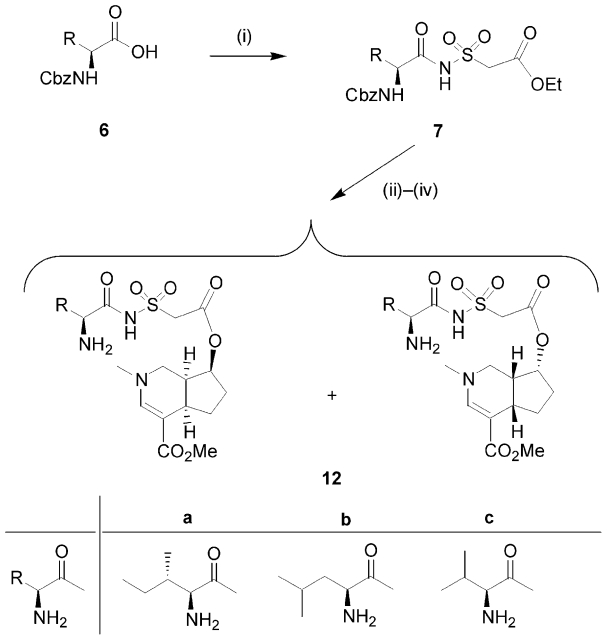
Scheme 1. (i) EDCI; $H_2NSO_2CH_2CO_2Et$, DMAP; (ii) NaOH, H_2O; (iii) ClCOCOCl, Et_3N; (±)-**3**, Et_3N, DMAP; (iv) 10% Pd/C, H_2.



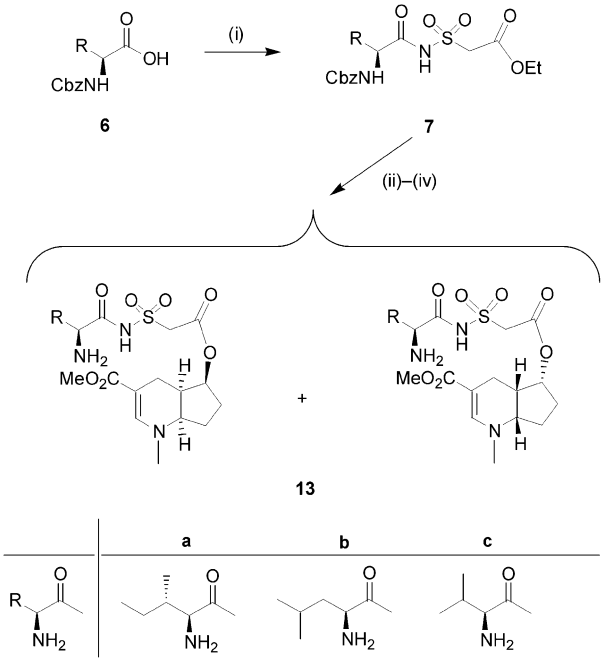
Compounds **8a–i**, **9a–e**, **11a–c**, **12a–c** and **13a–c** were evaluated as inhibitors of a range of tRNA synthetases and the results, along with those obtained earlier for SB-203207 (**1a**) and the semi-synthetic analogues **1b** and **1c**,[3] are summarised in Table 1. The isoleucine derivative SB-203207 (**1a**) is a potent inhibitor of IRS but less active against the corresponding leucyl and valyl synthetases (LRS and VRS). Compound **14** is an enzyme-bound intermediate in the reaction catalysed by IRS and the other tRNA synthetases involve similar intermediates, differing only in the amino acid side chains. It is reasonable to assume that **1a** inhibits IRS because it resembles the enzyme's intermediate **14**. One of the reasons for preparing **1b** and **1c** was because it therefore seemed likely that replacing the isoleucine residue of SB-203207 (**1a**) with, for example, leucine and valine, would produce the corresponding analogues of the intermediates of LRS and VRS, which would inhibit those enzymes. This hypothesis was substantiated through the selective inhibition of IRS, LRS and VRS by **1a–c**, respectively.[3] Our earlier studies showed that



Scheme 2. (i) EDCI; ethyl glycinate, ethyl β-alaninate or ethyl γ-aminobutyrate, DMAP; (ii) NaOH, H_2O; (iii) ClCOCOCl, Et_3N; (±)-**3**, Et_3N, DMAP; (iv) 10% Pd/C, H_2.



Scheme 3. (i) EDCI; $H_2NSO_2CH_2CO_2Et$, DMAP; (ii) NaOH, H_2O; (iii) ClCOCOCl, Et_3N; (±)-**4**, Et_3N, DMAP; (iv) 10% Pd/C, H_2.



Scheme 4. (i) EDCI; $H_2NSO_2CH_2CO_2Et$, DMAP; (ii) NaOH, H_2O; (iii) ClCOCOCl, Et_3N; (±)-**5**, Et_3N, DMAP; (iv) 10% Pd/C, H_2.

substituting the isoleucine side chain of **1a** with leucine in **1b** afforded a more potent inhibitor of LRS and a less potent inhibitor of either IRS or VRS. The analogous valine derivative **1c** was more potent than either **1a** or **1b** as an inhibitor of VRS, although it was also a potent IRS inhibitor.

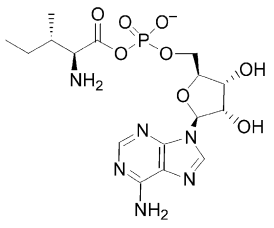
For this reason the synthetic compounds **8a**–**i**, **9a**–**e**, **11a**–**c**, **12a**–**c** and **13a**–**c** were evaluated as inhibitors of a range of synthetases, including those corresponding to their side chain amino acids. Except in the case of **8a**, they were only tested as ca. 1/1 mixtures of diastereomers, and any activity probably derives mostly from that stereoisomer most closely related to **1a**–**c** and **8a(I)**. Compounds **8a**–**i**, **9a**–**e**, **11a**–**c**, **12a**–**c** and **13a**–**c** are generally less active than **1a**–**c**. The vinylogous ureas **8a**–**c**, which are structurally the most similar to **1a**–**c**, are more active than the corresponding vinylogous carbamates **12a**–**c** and their isomers **13a**–**c**. Compounds **9a**–**c** and **11a**–**c**, which lack the terminal amino substituent and the sulfonyl group of the side chain of **1a**–**c**, respectively, show little or no inhibition. Changing either the carbon framework or the stereochemistry at either the α- or β-position of the isoleucine residue of **8a** results in the loss of enzyme inhibition with **8d**, **8e**, **8h** and **8i**.

Table 1. Inhibition of tRNA synthetases by SB-203207 (**1a**) and related compounds

Compd	Synthetase IC_{50} values (μM) or percentage inhibition at 100 μM[#]							
	IRS[a]	IRS[b]	LRS[a]	VRS[a]	ARS[a]	MRS[a]	PRS[a]	KRS[a]
1a	0.014	0.0042	1.55	0.126				
1b	0.91	0.068	0.016	0.29				
1c	0.037	0.0029	2.3	0.03				
8a(I)	3.7	0.57	0.42	6.35				
8a(II)	12.4	13.5	NI*	NI				
8b	NI	NI	2.4	NI				
8c	NI	NI	NI	NI				
8d	NI	NI	NI	NI				
8e	NI	NI	NI	NI				
8f	NI	NI	NI	NI	NI	55%	11%	
8g	38%	31%	13%	NI	NI	1.45	8%	
8h	46%	17%	18%	NI				
8i	NI	NI	NI	NI				
9a	NI	NI	NI	NI				
9b	NI	NI	NI	NI				
9c	NI	NI	NI	NI				
9d	NI	NI	NI	NI	NI	30%	68%	
9e	NI	NI	NI	NI				65%
11a	NI	17%	NI	NI				
11b	NI	10%	NI	NI				
11c	26%	18%	NI	NI				
12a	31%	49%	NI	NI				
12b	NI	14%	4.9	NI				
12c	38%	31%	13%	NI				
13a	34%	46%	NI	NI				
13b	15%	13%	24	NI				
13c	10%	33%	NI	10%				

[#]Determined according to the method reported previously.[5]
*NI, no inhibition detected.
[a]From *Staphylococcus aureus* WCUH29.
[b]From rat liver.

The correlation between the amino acid side chain and the selectivity of tRNA synthetase inhibition is confirmed by the present study. Of **8a**–**c**, **12a**–**c** and **13a**–**c**, those having leucine residues in the side chain (**8b**, **12b** and **13b**) inhibit LRS in preference to either IRS or VRS. Those containing isoleucine (**8a**, **12a** and **13a**) are generally more potent as inhibitors of IRS than of either LRS or VRS. The valine derivatives **8c**, **12c** and **13c** show no significant inhibition of VRS but they are generally less potent than either **8a**, **12a** or **13a**, or **8b**, **12b** or **13b**, as inhibitors of IRS and LRS, respectively.



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The derivatives of lysine **9e**, proline **9d** and alanine **8f** were prepared and tested to further examine this hypothesis. Consistent with this concept, the lysine derivative **9e** inhibits lysyl tRNA synthetase (KRS) but not IRS, LRS or VRS, and the proline derivative **9d** inhibits the proline synthetase (PRS) but not IRS, LRS, VRS or the alanine synthetase (ARS). The alanine derivative **8f** does not inhibit ARS but neither does it show significant inhibition of IRS, LRS, VRS or PRS. Somewhat unexpectedly, the alanine and proline derivatives **8f** and **9d** also display inhibition of methionyl tRNA synthetase (MRS). This implied that the methionine derivative **8g** would be a more potent MRS inhibitor. When it was made and tested it was found that, of the synthetic compounds **8a**–**i**, **9a**–**e**, **11a**–**c**, **12a**–**c** and **13a**–**c**, only **8a(I)** is more potent than **8g** against the corresponding synthetases, and **8g** is more selective. It is at least 50–100 times more potent against MRS than against either IRS, LRS, VRS, ARS or PRS.

Thus, replacing the isoleucine residue of the IRS inhibitor **8a(I)** with methionine in **8g** produced a selective MRS inhibitor. In a similar manner, Creppy et al.,[6,7] have reported that whereas ochratoxin A is an inhibitor of a phenylalanyl tRNA synthetase, substituting the phenylalanine residue of this compound with valine afforded a VRS inhibitor. Consequently this approach of amino acid substitution appears to be useful in the search for new and selective synthetase inhibitors.

Experimental

Melting points were determined on a Kofler hot-stage apparatus, equipped with a Reichert microscope, and are uncorrected. NMR spectra were recorded on either a Varian Inova 500 or a Varian Gemini 300 spectrometer, using the solvent systems specified. IR spectra were recorded using neat liquids or KBr disks, on either a Perkin–Elmer 1800 Fourier Transform Infrared spectrophotometer or a Perkin–Elmer 683 spectrophotometer. EIMS and HREIMS were recorded on either a VG Micromass 7070F or an AEI MS-30 spectrometer, operating at an ionisation potential of 70 eV. ESMS were obtained on a VG Quattro II mass spectrometer, operating with a cone voltage of 50 V. LSIMS-HRMS were recorded by the Central Science Laboratory, University of Hobart, using a Kratos Analytical Concept ISQ instrument, and *m*-nitrobenzyl alcohol as the matrix. HPLC was carried out using Waters™ 510HPLC pumps and the eluant was monitored using a Waters™ 486 Tunable Absorbance detector. A YMC-pack ODS-AQ, 250×10 mm, S-5 μm, 120 Å column was used, with the solvents indicated [A = 0.1% AcOH in H_2O, B = 0.1% AcOH in CH_3CN/water (9/1)], with detection at 254 or 300 nm) and a flow rate of 3 mL min^{-1}, unless otherwise indicated. Elemental analyses were performed by the Microanalytical Laboratory, Research School of Chemistry, the Australian National University.

The procedures used to prepare compound **8a** from **6a** are representative of those used to obtain **8b**–**i**, **9a**–**e**, **11a**–**c**, **12a**–**c** and **13a**–**c**.

Ethyl [[(2*S*,3*S*)-2-Benzyloxycarbonylamino-3-methyl-1-oxopentyl]amino]sulfonyl]acetate (7a). To a solution of DCC or EDCI (3 mmol) in DCM (50 mL) was added (2*S*,3*S*)-*N*-benzyloxycarbonylisoleucine (**6a**) (2.9 mmol). Ethyl 2-sulfamylacetate (3 mmol) and DMAP (8.85 mmol) were then added, and the mixture was heated at reflux for 24 h. It was then diluted with EtOAc (150 mL), washed with 1 M HCl (3×50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Column chromatography of the residue on silica, eluting with hexanes/EtOAc/AcOH (50/50/1, v/v/v), provided the title compound **7a**, yield 94%; oil; HPLC R_t 7.7 min (gradient of solvents A/B, 20/80→0/100 over 30 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.11 (s, 1H), 7.36 (m, 5H), 5.47 (d, J = 9.5 Hz, 1H), 5.18 and 5.12 (AB_q, J = 12.0 Hz, 2H), 4.39 (m, 1H), 4.43 and 4.34 (AB_q, J = 15.5 Hz, 2H), 4.19 (q, J = 7.0 Hz, 2H), 1.92 (m, 1H), 1.51 (m, 1H), 1.25 (t, J = 7.0 Hz, 3H), 1.13 (m, 1H), 0.99 (d, J = 7.0 Hz, 3H), 0.90 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 162.4, 156.9, 135.7, 128.5, 128.3, 128.0, 67.7, 62.6, 59.7, 56.0, 37.5, 24.2, 15.4, 13.8, 11.2; IR (neat) 3373, 3139, 2968, 2937, 1742, 1693, 1526, 1455, 1358, 1284, 1229, 1135, 1027, 909, 861, 734, 698 cm^{-1}; EI *m*/*z* 414 (M^+, 1%), 369 (2), 325 (2), 307 (10), 250 (6), 221 (18), 220 (53), 177 (16), 176 (53), 107 (16), 100 (19), 92 (21), 91 (100), 65 (15); EI-HRMS *m*/*z* 414.1461. calcd for $C_{18}H_{26}N_2O_7S$ (M^+) *m*/*z* 414.1461; Found: C, 51.54; H, 6.57; N, 6.88. calcd for $C_{18}H_{26}N_2O_7S.0.3H_2O$: C, 51.49; H, 6.39; N, 6.67%.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(2*S*,3*S*)-2-Amino-3-methyl-1-oxopentyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (8a). A solution of the ethyl ester (**7a**) (0.52 mmol) in ethanol (5 mL) and NaOH (3 mL, 1 M aq) was stirred for 1 h, before it was adjusted to pH 3 with HCl (2 M, aq). The mixture was concentrated under reduced pressure and the resulting suspension was extracted with ethyl acetate (3×4 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The residual white solid was stored in a dessicator overnight over P_2O_5, and then used without further purification. The solid was added to a mixture of triethylamine (0.44 mmol) in DCM, maintained at 0 °C. Then oxalyl chloride (0.46 mmol) was added, and that mixture was stirred for 30 min. A solution of the bicyclic alcohol (±)-3 (0.25 mmol), triethylamine (0.22 mmol) and DMAP (3 mg) in DMF (1.5 mL) was then added dropwise to the mixture over 1 min. After stirring for 1 h, EtOH (2 mL) was added and

the mixture was allowed to warm to room temperature before it was concentrated under reduced pressure. The residue was diluted with water (4 mL) and extracted with ethyl acetate (5×2 mL). The organic extracts were concentrated under reduced pressure and the residue was chromatographed on silica, eluting with AcOH/EtOH/EtOAc (0.1/30/70, v/v/v), to yield a ca. 1/1 mixture of diastereomers of the benzyloxycarbonyl derivative of **8a** as a colourless solid (47%), mp 110–113 °C; HPLC R_t 11.1 min (gradient of solvents A/B, 30/70→ 0/100 over 10 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.42 (m, 6H), 5.49 (m, 1H), 5.17 (m, 2H), 4.57 (m, 2H), 4.18 and 4.17 (d and d, J = 7.0 and 7.0 Hz, total 1H), 3.16 (dd, J = 5.5, 13.0 Hz, 1H), 3.08 and 3.07 (s and s, total 3H), 2.96 (m, 1H), 2.78 (m, 1H), 2.48 (m, 1H), 2.27 (m, 2H), 1.91 (m, 2H), 1.61 (m, 1H), 1.44 (m, 1H), 1.28 (m, 1H), 1.08 (d, J = 6.5 Hz, 3H), 1.00 (t, J = 7.0 Hz, 3H); IR (neat) 3509, 3379, 3055, 2968, 2878, 1722, 1642, 1518, 1395, 1346, 1265, 1139, 1074, 896, 870, 737, 703 cm^{-1}; ESMS (+ve) m/z 587 ($M+Na^+$), 603 ($M+K^+$); Found: C, 55.16; H, 6.47; N, 9.80. calcd for $C_{26}H_{36}N_4O_8S$: C, 55.31; H, 6.43; N, 9.92%.

The benzyloxycarbonyl derivative of **8a** was added to a suspension of 10% Pd/C (5 mg) in MeOH (3 mL), and the mixture was stirred under an atmosphere of hydrogen overnight at room temperature, before it was filtered through Celite and the filtrate was concentrated under reduced pressure. HPLC of the residue afforded a ca. 1/1 mixture of diastereomers of the title compound **8a** (84%) after freeze-drying from dilute aqueous hydrochloric acid, mp 192 °C (dec.); HPLC R_t 11.4 min (gradient of solvents A/B, 100/0→ 40/60 over 11 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.92 (s, 1H), 5.46 (m, 1H), 4.59 (s, 2H), 3.93 (d, J = 3.5 Hz, 1H), 3.33 (m, 1H), 3.24 (s, 3H), 3.20 (m, 1H), 2.78 (m, 1H), 2.52 (m, 1H), 2.24 (m, 2H), 2.06 (m, 1H), 1.85 (m, 1H), 1.48 (m, 2H), 1.23 (m, 1H), 1.12 (d, J = 7.0 Hz, 3H), 0.99 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 170.9, 170.1, 170.0, 163.7, 152.5, 92.4, 79.7, 59.7, 57.8, 57.7, 45.4, 44.5, 38.5, 37.7, 37.6, 32.9, 30.1, 29.0, 28.9, 24.8, 24.7, 15.4, 11.8; IR (KBr) 3327, 2968, 1740, 1680, 1625, 1587, 1464, 1416, 1357, 1282, 1192, 1153, 1080, 899, 852, 509 cm^{-1}; LSIMS-HRMS m/z 431.1980. calcd for $C_{18}H_{31}N_4O_6S$ ($M+H^+$) m/z 431.1964; Found: C, 41.60; H, 7.35; N, 10.63. calcd for $C_{18}H_{30}N_4O_6S.3H_2O.HCl$: C, 41.49; H, 7.16; N, 10.75%.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(*S*)-2-Amino-4-methyl-1-oxopentyl]amino]sulfonyl]acetyloxy] - 2,4a,5,6,7,7a - hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (8b). Isolated by freeze-drying from acetic acid solution, mp 159–165 °C; HPLC R_t 11.5 min (gradient of solvents A/B, 100/0→0/100 over 20 min); ^{1}H NMR (500 MHz, CD_3OD) δ 7.42 (s, 1H), 5.48 (m, 1H), 4.31 (m, 2H), 3.72 (m, 1H), 3.26 (dd, J = 13.0, 5.5 Hz, 1H), 3.11 (s, 3H), 2.98 (m, 1H), 2.80 (m, 1H), 2.50 (m, 1H), 2.28 (m, 2H), 1.91 (m, 3H), 1.71 (m, 1H), 1.45 (m, 1H), 1.08 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 176.8, 174.0, 166.0, 165.9, 146.0, 100.7(0), 100.6(8), 79.3(0), 79.2(7), 57.0, 55.5(2), 55.5(1), 44.9, 43.1, 41.9, 39.3, 34.5, 31.5(9), 31.5(6), 28.8(4), 28.7(9), 25.6, 23.3, 22.0; IR (KBr) 3608, 3582, 3434, 3361, 3218, 2956, 1728, 1629, 1529, 1387, 1286, 1134, 1074, 845, 665 cm^{-1}; ESMS (−ve) m/z 429 ($M-H^+$); LSIMS-HRMS m/z 431.1954. calcd for $C_{18}H_{31}N_4O_6S$ ($M+H^+$) m/z 431.1964.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(*S*)-2-Amino-3-methyl-1-oxobutyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (8c). Isolated by freeze-drying from dilute aqueous hydrochloric acid, mp 230 °C (dec.); HPLC R_t 10.8 min (gradient of solvents A/B, 100/0→10/90 over 20 min); ^{1}H NMR (500 MHz, CD_3OD) δ 7.99 (s, 1H), 5.57 (m, 1H), 4.68 (m, 2H), 3.95 (m, 1H), 3.41 (m, 1H), 3.32 (s, 3H), 3.29 (m, 1H), 2.86 (m, 1H), 2.62 (m, 1H), 2.45 (m, 1H), 2.34 (m, 2H), 1.95 (m, 1H), 1.53 (m, 1H), 1.24 (d, J = 7.0 Hz, 3H), 1.15 and 1.14 (d and d, J = 7.0 and 7.0 Hz, total 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.0, 170.0(3), 170.0(0), 163.7, 152.4, 92.6, 79.7, 60.0, 57.7, 45.3, 44.4, 38.6, 33.0, 31.2, 31.1, 30.2, 29.0, 28.9, 19.1, 19.0, 16.9; IR (KBr) 3430, 2982, 2921, 1737, 1627, 1463, 1356, 1282, 1193, 1128 cm^{-1}; ESMS (−ve) m/z 415 ($M-H^+$); LSIMS-HRMS m/z 417.1800. calcd for $C_{17}H_{29}N_4O_6S$ ($M+H^+$) m/z 417.1808.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(2*S*,3*R*)-2-Amino-3-methyl-1-oxopentyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (8d). Isolated by freeze-drying from dilute aqueous hydrochloric acid, mp 190–210 °C (dec.); HPLC R_t 11.9 min (gradient of solvents A/B, 100/0→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 8.00 (s, 1H), 5.57 (m, 1H), 4.68 (s, 2H), 4.09 (m, 1H), 3.42 (m, 1H), 3.33 (s, 3H), 3.26 (m, 1H), 2.86 (m, 1H), 2.62 (m, 1H), 2.35 (m, 2H), 2.22 (m, 1H), 1.96 (m, 1H), 1.66 (m, 1H), 1.50 (m, 2H), 1.12 (t, J = 7.5 Hz, 3H), 1.09 (d, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 170.9, 170.3, 163.7, 152.4, 92.6, 79.7, 79.6, 58.9, 58.8, 57.6, 45.3, 44.5, 44.4, 38.6, 37.4, 37.3, 33.0, 30.2, 28.9, 27.1, 13.3, 12.0; IR (KBr) 3410, 2967, 1739, 1679, 1625, 1587, 1463, 1356, 1283, 1158, 852 cm^{-1}; ESMS (−ve) m/z 429 ($M-H^+$); LSIMS-HRMS m/z 431.1976. calcd for $C_{18}H_{31}N_4O_6S$ ($M+H^+$) m/z 431.1964; Found: C, 39.89; H, 6.51; N, 10.34. calcd for $C_{18}H_{30}N_4O_6S.3HCl$: C, 40.04; H, 6.16; N, 10.38%.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(2*R*,3*R*)-2-Amino-3-methyl-1-oxopentyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (8e). Isolated by freeze-drying from dilute aqueous hydrochloric acid, mp 193–208 °C (dec.); HPLC R_t 11.4 min (gradient of solvents A/B, 100/0→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.88 (s, 1H), 5.46 (m, 1H), 4.58 (s, 2H), 3.87 (d, J = 4.5 Hz, 1H), 3.34 (m, 1H), 3.22 (s, 3H), 3.16 (m, 1H), 2.74 (m, 1H), 2.51 (m, 1H), 2.25 (m, 2H), 2.07 (m, 1H), 1.88 (m, 1H), 1.56 (m, 1H), 1.42 (m, 1H), 1.21 (m, 1H), 1.11 (d, J = 7.0 Hz, 3H), 0.99 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 171.1, 170.1, 163.8, 152.2, 92.9, 79.7, 79.6, 59.9, 57.6, 45.3, 44.4, 38.6, 37.8, 37.7, 33.0, 30.2, 29.0, 24.7(4), 24.6(8), 15.5, 11.9; IR (KBr) 3430, 2975, 2926, 1740, 1626, 1463, 1356, 1281, 1156, 1118 cm^{-1}; ESMS (−ve) m/z 429 ($M-H^+$); LSIMS-HRMS m/z 431.1975. calcd for $C_{18}H_{31}N_4O_6S$ ($M+H^+$) m/z 431.1964.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(*S*)-2-Amino-1-oxopropyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (8f). Isolated by freeze-drying from acetic acid solution, mp 155–161 °C; HPLC R_t 9.9 min (gradient of solvents A/B, 100/0→60/40 over 13 min); ^{1}H NMR (500 MHz, CD_3OD) δ 7.42 (s, 1H), 5.48 (m, 1H), 4.32 (m, 2H), 3.77 (q, J = 7.0 Hz, 1H), 3.26 (m, 1H), 3.11 (s, 3H), 2.98 (m, 1H), 2.79 (m, 1H), 2.50 (m, 1H), 2.28 (m, 2H), 1.93 (m, 1H), 1.57 (d, J = 7.0 Hz, 3H), 1.45 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 177.2, 174.3, 166.3, 146.3, 101.0, 79.6, 58.5, 53.1, 45.2, 43.4, 39.6, 34.9, 31.9, 29.1, 17.9; IR (KBr) 3438, 3229, 2929, 1730, 1635, 1522, 1396, 1285, 1120, 765 cm^{-1}; ESMS (−ve) m/z 387 ($M-H^+$); LSIMS-HRMS m/z 389.1495. calcd for $C_{15}H_{25}N_4O_6S$ ($M+H^+$) m/z 389.1494.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(*S*)-2-Amino-1-oxo-5-thiahexyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (8g). Isolated by freeze-drying from acetic acid solution, mp 135–142 °C; HPLC R_t 11.5 min (gradient of solvents A/B, 100/0→ 0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.32 (s, 1H), 5.39 (m, 1H), 4.24 (m, 2H), 3.78 and 3.75 (d and d, J = 5.5 and 5.5 Hz, total 1H), 3.16 (dd, J = 13.0, 5.5 Hz, 1H), 3.01 (s, 3H), 2.88 (m, 1H), 2.66 (m, 3H), 2.41 (m, 1H), 2.19 (m, 3H), 2.11 (s, 3H), 2.06 (m, 1H), 1.82 (m, 1H), 1.37 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.1, 174.3, 166.3, 146.3, 101.0, 79.6, 57.8, 56.4, 45.2, 43.5, 39.6, 34.8(3), 34.8(0), 32.4, 31.9, 30.5, 29.2, 29.1, 15.3; IR (KBr) 3447, 2924, 1728, 1636, 1526, 1394, 1290, 1135, 1073, 848 cm^{-1}; ESMS (−ve) m/z 447 ($M-H^+$); LSIMS-HRMS m/z 449.1525. calcd for $C_{17}H_{29}N_4O_6S_2$ ($M+H^+$) m/z 449.1528.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(*S*)-2-Amino-1-oxohexyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (8h). Isolated by freeze-drying from dilute aqueous hydrochloric acid, mp 152–153 °C; HPLC R_t 11.8 min (gradient of solvents A/B, 100/0→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.32 (s, 1H), 5.38 (m, 1H), 4.23 (m, 2H), 3.58 (m, 1H), 3.17 (dd, J = 5.5, 13.0 Hz, 1H), 3.01 (s, 3H), 2.88 (m, 1H), 2.70 (m, 1H), 2.40 (m, 1H), 2.18 (m, 2H), 1.84 (m, 3H), 1.39 (m, 5H), 0.94 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.6, 174.3, 166.1(8), 166.2(3), 146.3, 101.0, 79.6, 57.7, 57.3, 45.2, 43.4, 39.6, 34.9, 32.7, 31.9, 29.2, 28.4, 23.9, 14.5; IR (KBr) 3439, 2957, 2926, 1729, 1638, 1539, 1291, 1118, 1074, 851 cm^{-1}; ESMS (−ve) m/z 429 ($M-H^+$); LSIMS-HRMS m/z 431.1964. calcd for $C_{18}H_{31}N_4O_6S$ ($M+H^+$) m/z 431.1966.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(*S*)-2-Amino-1-oxopentyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (8i). Isolated by freeze-drying from dilute aqueous hydrochloric acid, mp 190–210 °C (dec.); HPLC R_t 11.3 min (gradient of solvents A/B, 100/0→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 8.01 (s, 1H), 5.57 (m, 1H), 4.67 (s, 2H), 4.11 (m, 1H), 3.41 (m, 1H), 3.33 (s, 3H), 3.26 (m, 1H), 2.86 (m, 1H), 2.62 (m, 1H), 2.34 (m, 2H), 1.98 (m, 3H), 1.55 (m, 3H), 1.11 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 170.9, 170.6, 163.7, 152.4, 92.5, 79.7, 79.6, 57.6, 57.5, 55.0, 54.9, 45.4, 44.5, 44.4, 38.6, 34.0, 33.9, 33.0, 30.2, 29.0, 18.9, 14.0; IR (KBr) 3418, 2965, 1738, 1678, 1625, 1464, 1355, 1282, 1152, 865 cm^{-1}; ESMS (−ve) m/z 415 ($M-H^+$); LSIMS-HRMS m/z 417.1788. calcd for $C_{17}H_{29}N_4O_6S$ ($M+H^+$) m/z 417.1809.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(*S*)-3-Methyl-1-oxopentyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (9a). Mp 95–98 °C; HPLC R_t 10.5 min (gradient of solvents A/B, 55/45→0/100 over 20 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.36 (s, 1H), 5.69 (bs, 2H), 5.37 (m, 1H), 4.43 (AB_q, J = 17.0 Hz, 2H), 3.00 (s, 3H), 2.97 (m, 1H), 2.88 (m, 1H), 2.58 (m, 1H), 2.42 (m, 2H), 2.17 (m, 2H), 1.96 (m, 1H), 1.79 (m, 1H), 1.41 (m, 2H), 1.26 (m, 1H), 0.91 (d, J = 7.5 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 171.4, 162.5, 145.4, 98.4, 78.8, 56.4, 43.8, 43.5, 43.1, 37.8, 33.1, 31.8, 30.3, 29.4, 27.8, 19.2, 11.3; IR (neat) 3482, 3377, 3221, 2961, 2875, 1739, 1642, 1538, 1461, 1400, 1344, 1287, 1144, 1119, 1075, 909, 865, 755 cm^{-1}; ESMS (−ve) m/z 414 ($M-H^+$); LSIMS-HRMS m/z 416.1847. calcd for $C_{18}H_{30}N_3O_6S$ ($M+H^+$) m/z 416.1855; Found: C, 51.62; H, 7.35; N, 10.06. calcd for $C_{18}H_{29}N_3O_6S$: C, 52.03; H, 7.03; N, 10.11%.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[4-Methyl-1-oxopentyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (9b). Mp 115–117 °C; HPLC R_t 9.7 min (gradient of solvents A/B, 55/45→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.32 (s, 1H), 5.42 (m, 1H), 4.45 (m, 2H), 3.08 (m, 1H), 3.00 (s, 3H), 2.88 (m, 1H), 2.69 (m, 1H), 2.42 (m, 1H), 2.33 (m, 2H), 2.20 (m, 2H), 1.81 (m, 1H), 1.53 (m, 3H), 1.36 (m, 1H), 0.91 (d, J = 6.5 Hz, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.4, 173.9, 164.0, 145.9, 100.8, 79.9, 57.0, 44.8, 43.1, 39.2, 35.3, 34.5, 31.6, 28.9, 28.8, 22.6; IR (KBr) 3369, 2952, 2868, 1734, 1638, 1542, 1467, 1386, 1343, 1287, 1140, 1118, 1074, 873 cm^{-1}; ESMS (+ve) m/z 399 ($M-NH_3+H^+$), 416 ($M+H^+$), 438 ($M+Na^+$); LSIMS-HRMS m/z 416.1840. calcd for $C_{18}H_{30}N_3O_6S$ ($M+H^+$) m/z 416.1855; Found: C, 52.19; H, 6.96; N, 9.72. calcd for $C_{18}H_{29}N_3O_6S$: C, 52.03; H, 7.03; N, 10.11%.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[3-Methyl-1-oxobutyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (9c). Mp 125–132 °C; HPLC R_t 7.6 min (gradient of solvents A/B, 55/45→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.32 (s, 1H), 5.42 (m, 1H), 4.47 (m, 2H), 3.08 (m, 1H), 3.00 (s, 3H), 2.88 (m, 1H), 2.71 (m, 1H), 2.40 (m, 1H), 2.18 (m, 5H), 1.82 (m, 1H), 1.38 (m, 1H), 0.98 (d, J = 6.5 Hz, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.7, 173.9, 164.0, 146.0, 100.8, 79.9, 57.0, 46.2, 44.8, 43.1, 39.2, 34.5, 31.6, 28.9, 26.8, 22.7; IR (KBr) 3458, 3376, 3213, 2959, 2872, 1737, 1641, 1547, 1345, 1289, 1146, 1119, 1075, 904, 865 cm^{-1}; ESMS (+ve) m/z 385 ($M-NH_3+H^+$), 402 ($M+H^+$); LSIMS-HRMS m/z 402.1686. calcd for $C_{17}H_{28}N_3O_6S$ ($M+H^+$) m/z 402.1699.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(*S*)-Pyrrolidin-2-oyl]-amino]sulfonyl]acetyloxy] - 2,4a,5,6,7,7a - hexahydro - 2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (9d). Isolated by freeze-drying from acetic acid solution, mp 148–150 °C; HPLC R_t 10.5 min (gradient of solvents A/B, 100/0→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.42 (s, 1H), 5.49 (m, 1H), 4.32 (m, 2H), 4.16 (m, 1H), 3.49 (m, 1H), 3.37 (m, 1H), 3.29 (m, 1H), 3.11 (s, 3H), 2.98 (m, 2H), 2.79 (m, 1H), 2.45 (m, 2H), 2.29 (m, 3H), 2.10 (m, 1H), 1.90 (m, 1H), 1.45 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.4, 174.0, 165.9, 146.1, 146.0, 100.7, 79.3(1), 79.2(5), 63.7, 47.3, 44.9, 44.8, 43.2, 43.1, 39.4, 39.3, 34.5(3), 34.4(7), 31.6(2), 31.5(6), 30.7, 29.0, 28.8, 24.9; IR (KBr) 3608, 3582, 3438, 3368, 2946, 1727, 1628, 1558, 1390, 1286, 1135, 1074, 845 cm^{-1}; ESMS (−ve) *m/z* 413 (M−H$^+$); LSIMS-HRMS *m/z* 415.1628. calcd for $C_{17}H_{27}N_4O_6S$ (M+H$^+$) *m/z* 415.1651; Found: C, 46.66; H, 6.27; N, 12.54. calcd for $C_{17}H_{26}N_4O_6S.1.5H_2O$: C, 46.25; H, 6.62; N, 12.69%.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(*S*)-2,6-Diamino-1-oxohexyl]amino]sulfonyl]acetyloxy] - 2,4a,5,6,7,7a - hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (9e). Isolated by freeze-drying from dilute aqueous hydrochloric acid, mp 126–129 °C; HPLC R_t 9.3 min (gradient of solvents A/B, 100/0→50/50 over 10 min); ^{1}H NMR (300 MHz, CD_3OD) δ 8.02 (s, 1H), 5.58 (m, 1H), 4.72 and 4.66 (AB_q, J=14.5 Hz, 2H), 4.18 (m, 1H), 3.41 (m, 1H), 3.34 (s, 3H), 3.28 (m, 1H), 3.08 (t, J=7.5 Hz, 2H), 2.88 (m, 1H), 2.61 (m, 1H), 2.35 (m, 2H), 2.04 (m, 3H), 1.86 (m, 2H), 1.64 (m, 2H), 1.57 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 171.2, 170.6, 164.1(2), 164.0(7), 152.8, 92.7, 80.0, 57.9, 57.8, 55.0, 45.7, 44.8, 40.5, 38.8, 33.3, 31.7, 31.6, 30.5, 29.3, 28.3, 22.9; IR (KBr) 3608, 3582, 3350, 3235, 2943, 1728, 1630, 1549, 1401, 1286, 1133, 848 cm^{-1}; ESMS (−ve) *m/z* 444 (M−H$^+$); LSIMS-HRMS *m/z* 446.2080. calcd for $C_{18}H_{32}N_5O_6S$ (M+H$^+$) *m/z* 446.2073.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(2*S*,3*S*)-2-Amino-3-methyl-1-oxopentyl]amino]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (11a). Isolated by freeze-drying from acetic acid solution, mp > 150 °C (dec.); HPLC R_t 13.0 and 13.5 min (gradient of solvents A/B, 100/0→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.31 (s, 1H), 5.39 (m, 1H), 4.01 (m, 2H), 3.56 (d, J=5.5 Hz, 1H), 3.18 (m, 1H), 3.00 (s, 3H), 2.89 (m, 1H), 2.71 (m, 1H), 2.43 (m, 1H), 2.17 (m, 2H), 1.86 (m, 2H), 1.59 (m, 1H), 1.30 (m, 2H), 1.04 (d, J=7.0 Hz, 3H), 0.97 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.0, 173.0, 172.9, 170.9(2), 170.8(6), 145.9, 101.1, 79.2, 59.7, 45.0, 43.1, 42.0, 39.5, 39.4, 38.9, 34.4(1), 34.3(6), 31.6, 29.1, 29.0, 25.5, 15.4, 11.8; IR (KBr) 3325, 3216, 2961, 2930, 1735, 1643, 1550, 1458, 1384, 1343, 1285, 1198, 1118, 1074, 1033, 768 cm^{-1}; ESMS (+ve) *m/z* 350 (M−NH$_3$+H$^+$) 367 (M+H$^+$); LSIMS-HRMS *m/z* 367.2331. calcd for $C_{18}H_{31}N_4O_4$ (M+H$^+$) *m/z* 367.2345.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[3-[(2*S*,3*S*)-2-Amino-3-methyl-1-oxopentyl]amino]propanoyl]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (11b). Isolated by freeze-drying from acetic acid solution, mp > 150 °C (dec.); HPLC R_t 15.6 min (gradient of solvents A/B, 100/0→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ7.31 (s, 1H), 5.35 (m, 1H), 3.55 (m, 1H), 3.45 (m, 2H), 3.00 (s, 3H), 2.98 (m, 1H), 2.89 (d, J=12.0 Hz, 1H), 2.71 (m, 1H), 2.59 (m, 2H), 2.38 (m, 1H), 2.18 (m, 2H), 1.77 (m, 2H), 1.53 (m, 1H), 1.36 (m, 1H), 1.18 (m, 1H), 0.95 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.9, 172.9, 172.3, 145.9, 101.1, 78.3, 59.5, 45.0, 43.2, 43.1, 39.4, 38.8, 36.4, 34.7, 34.3, 31.6, 29.1, 25.6, 15.4, 11.8; IR (KBr) 3344, 3225, 3069, 2964, 2944, 2877, 1733, 1646, 1557, 1389, 1340, 1283, 1182, 1117, 1074, 769 cm^{-1}; ESMS (+ve) *m/z* 364 (M−NH$_3$+H$^+$), 381 (M+H$^+$); LSIMS-HRMS *m/z* 381.2485. calcd for $C_{19}H_{33}N_4O_4$ (M+H$^+$) *m/z* 381.2501.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[4-[(2*S*,3*S*)-2-Amino-3-methyl-1-oxopentyl]amino]butanoyl]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxamide (11c). Isolated by freeze-drying from acetic acid solution, mp > 150 °C (dec.); HPLC R_t 13.2 and 13.3 min (gradient of solvents A/B, 100/0→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.31 (s, 1H), 5.34 (m, 1H), 3.46 (m, 1H), 3.29 (m, 2H), 3.00 (s, 3H), 2.92 (m, 2H), 2.70 (m, 1H), 2.38 (m, 3H), 2.17 (m, 2H), 1.83 (m, 3H), 1.74 (m, 1H), 1.56 (m, 1H), 1.36 (m, 1H), 1.20 (m, 1H), 0.96 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.4, 173.9, 172.3, 145.9, 101.1, 78.2, 59.7, 45.1, 43.1, 39.7, 39.3, 38.9, 34.4, 32.3, 31.6, 29.2, 25.7, 25.6, 15.5, 11.8; IR (KBr) 3339, 3227, 3072, 2966, 2934, 2877, 1732, 1646, 1557, 1401, 1340, 1284, 1174, 1118, 1075, 880, 769, 651 cm^{-1}; ESMS (+ve) *m/z* 378 (M−NH$_3$+H$^+$), 395 (M+H$^+$), 417 (M+Na$^+$); LSIMS-HRMS *m/z* 395.2647. calcd for $C_{20}H_{35}N_4O_4$ (M+H$^+$) *m/z* 395.2658.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(2*S*,3*S*)-2-Amino-3-methyl-1-oxopentyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxylic acid methyl ester (12a). Isolated by freeze-drying from acetic acid solution, mp 116–120 °C; HPLC R_t 12.6 min (gradient of solvents A/B, 60/40→0/100 over 20 min); ^{1}H NMR (500 MHz, CD_3OD) δ 7.53 (s, 1H), 5.47 (m, 1H), 4.32 (m, 2H), 3.72 (s, 3H), 3.63 (m, 1H), 3.28 (dd, J=13.0, 6.0 Hz, 1H), 3.12 (s, 3H), 3.00 (m, 1H), 2.84 (m, 1H), 2.47 (m, 1H), 2.24 (m, 2H), 2.10 (m, 1H), 1.91 (m, 1H), 1.73 (m, 1H), 1.44 (m, 1H), 1.35 (m, 1H), 1.14 (d, J=7.5 Hz, 3H), 1.06 (m, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 175.5, 175.4, 171.3, 165.9(2), 165.8(7), 148.4, 98.2(4), 98.2(3), 79.4(0), 79.3(9), 61.6, 61.5, 57.3, 51.0, 45.0(4), 45.0(3), 43.2, 39.0, 38.0, 34.7, 31.7, 28.9, 28.8, 25.5(0), 25.4(6), 15.4(8), 15.4(6), 12.2; IR (KBr) 3608, 3582, 3492, 3119, 2956, 1730, 1617, 1486, 1259, 1177, 1129, 1076, 843 cm^{-1}; ESMS (+ve) *m/z* 414 (M−MeOH+H$^+$), 446 (M+H$^+$); LSIMS-HRMS *m/z* 446.1927. calcd for $C_{19}H_{32}N_3O_7S$ (M+H$^+$) *m/z* 446.1960.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(*S*)-2-Amino-4-methyl-1-oxopentyl]amino]sulfonyl]acetyloxy] - 2,4a,5,6,7,7a - hexahydro - 2 - methyl - 1*H* - cyclopenta[c]pyridine - 4 - carboxylic acid methyl ester (12b). Isolated by freeze-drying from acetic acid solution, mp 119–122 °C; HPLC R_t 11.2 min

(gradient of solvents A/B, 80/20→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.32 (s, 1H), 5.27 (m, 1H), 4.11 (m, 2H), 3.51 (s, 3H), 3.49 (m, 1H), 3.07 (dd, J = 13.0, 5.0 Hz, 1H), 2.92 (s, 3H), 2.80 (m, 1H), 2.63 (m, 1H), 2.26 (m, 1H), 2.04 (m, 2H), 1.74 (m, 3H), 1.53 (m, 1H), 1.23 (m, 1H), 0.89 (d, J = 5.0 Hz, 3H), 0.86 (d, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.7, 171.3, 165.9(4), 165.8(9), 148.4, 98.2, 79.4, 56.9, 55.5, 51.0, 45.0, 43.2(3), 43.1(6), 42.0, 39.0, 34.7, 31.7, 28.9, 25.6, 23.3, 22.0; IR (KBr) 3604, 3582, 2954, 1731, 1666, 1615, 1438, 1410, 1287, 1260, 1177, 1134, 1075, 845 cm^{-1}; ESMS (−ve) m/z 444 ($M-H^+$); LSIMS-HRMS m/z 446.1973. calcd for $C_{19}H_{32}N_3O_7S$ ($M+H^+$) m/z 446.1960.

(4a*R*,7*S*,7a*S*)- and (4a*S*,7*R*,7a*R*)-7-[[(*S*)-2-Amino-3-methyl-1-oxobutyl]amino]sulfonyl]acetyloxy]-2,4a,5,6,7,7a-hexahydro-2-methyl-1*H*-cyclopenta[c]pyridine-4-carboxylic acid methyl ester (12c). Isolated by freeze-drying from dilute aqueous hydrochloric acid, mp 150–165 °C; HPLC R_t 14.1 min (gradient of solvents A/B, 100/0→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.43 (s, 1H), 5.42 (m, 1H), 4.56 (s, 2H), 3.84 (m, 1H), 3.62 (s, 3H), 3.11 (m, 1H), 3.02 (s, 3H), 2.91 (m, 1H), 2.75 (m, 1H), 2.36 (m, 2H), 2.16 (m, 2H), 1.80 (m, 1H), 1.36 (m, 1H), 1.13 (d, J = 7.0 Hz, 3H), 1.04 (d, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 171.2, 170.0, 169.9, 163.9, 148.3, 98.4, 80.4(0), 80.3(5), 60.0, 57.4, 51.1, 45.0, 43.2(2), 43.1(6), 39.0, 34.6, 31.7, 31.2, 31.1, 28.9, 19.1(1), 19.0(6), 16.8; IR (KBr) 3604, 3582, 2920, 2848, 1733, 1615, 1460, 1356, 1302, 1273, 1147, 1126, 1075 cm^{-1}; ESMS (−ve) m/z 430 ($M-H^+$); LSIMS-HRMS m/z 432.1784. calcd for $C_{18}H_{30}N_3O_7S$ ($M+H^+$) m/z 432.1804.

(4a*R*,5*S*,7a*R*)- and (4a*S*,5*R*,7a*S*)-7-[[(2*S*,3*S*)-2-Amino-3-methyl-1-oxopentyl]amino]sulfonyl]acetyloxy]-4,4a,5,6,7,7a-hexahydro-1-methyl-1*H*-cyclopenta[b]pyridine-3-carboxylic acid methyl ester (13a). Isolated by freeze-drying from acetic acid solution, mp 130–135 °C; HPLC R_t 8.4 min (gradient of solvents A/B, 80/20→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.31 (s, 1H), 5.16 (m, 1H), 4.20 (m, 1H), 4.05 (m, 1H), 3.51 (s, 3H), 3.49 (m, 1H), 3.32 (m, 1H), 2.90 (s, 3H), 2.22 (m, 2H), 1.90 (m, 5H), 1.46 (m, 2H), 1.13 (m, 1H), 0.92 (d, J = 7.0 Hz, 3H), 0.83 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.7, 171.6, 166.3, 147.8(9), 147.8(6), 92.9, 92.8, 78.6(4), 78.6(0), 61.8(3), 61.7(6), 59.5, 57.8, 51.4, 42.1, 38.4, 38.3, 28.3, 27.6, 27.5, 25.8, 25.7, 18.0, 15.8, 12.5; IR (KBr) 3608, 3582, 3468, 2962, 1731, 1615, 1442, 1415, 1383, 1325, 1271, 1180, 1131, 1075, 843, 762 cm^{-1}; ESMS (+ve) m/z 414 ($M-MeOH+H^+$), 446 ($M+H^+$), 468 ($M+Na^+$); LSIMS-HRMS m/z 446.1954. calcd for $C_{19}H_{32}N_3O_7S$ ($M+H^+$) m/z 446.1960.

(4a*R*,5*S*,7a*R*)- and (4a*S*,5*R*,7a*S*)-7-[[(*S*)-2-Amino-4-methyl-1-oxopentyl]amino]sulfonyl]acetyloxy] - 4,4a,5,6,7,7a - hexahydro - 1 - methyl - 1*H* - cyclopenta[b]pyridine - 3 - carboxylic acid methyl ester (13b). Isolated by freeze-drying from acetic acid solution, mp 129–131 °C; HPLC R_t 11.2 min (gradient of solvents A/B, 80/20→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.32 (s, 1H), 5.17 (m, 1H), 4.20 (m, 1H), 4.08 (m, 1H), 3.56 (m, 1H), 3.52 (s, 3H), 3.33 (m, 1H), 2.92 (s, 3H), 2.24 (m, 2H), 2.02 (m, 2H), 1.75 (m, 4H), 1.47 (m, 2H), 0.87 (br, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.8, 171.3, 166.0, 147.6(2), 147.5(7), 92.5(2), 92.4(9), 78.3, 59.2, 57.1, 55.5, 51.2, 41.9(2), 41.8(7), 38.0, 28.1, 27.2(4), 27.1(7), 25.6, 23.4, 21.9, 17.8; IR (KBr) 3604, 3582, 2953, 1731, 1614, 1438, 1413, 1381, 1298, 1273, 1180, 1135, 1110, 845 cm^{-1}; ESMS (−ve) m/z 444 ($M-H^+$); LSIMS-HRMS m/z 446.1945. calcd for $C_{19}H_{32}N_3O_7S$ ($M+H^+$) m/z 446.1960.

(4a*R*,5*S*,7a*R*)- and (4a*S*,5*R*,7a*S*)-7-[[(*S*)-2-Amino-3-methyl-1-oxobutyl]amino]sulfonyl]acetyloxy] - 4,4a,5,6,7,7a - hexahydro - 1 - methyl - 1*H* - cyclopenta[b]pyridine - 3 - carboxylic acid methyl ester (13c). Isolated by freeze-drying from dilute aqueous hydrochloric acid, mp 135–160 °C; HPLC R_t 14.0 min (gradient of solvents A/B, 100/0→0/100 over 20 min); ^{1}H NMR (300 MHz, CD_3OD) δ 7.44 (s, 1H), 5.35 (m, 1H), 4.58 (m, 2H), 4.04 and 4.00 (d and d, J = 4.0 and 4.0 Hz, total 1H), 3.77 (m, 1H), 3.64 (s, 3H), 3.46 (m, 1H), 3.03 (s, 3H), 2.36 (m, 2H), 2.16 (m, 2H), 1.93 (m, 2H), 1.58 (m, 1H), 1.15 and 1.14 (d and d, J = 7.0 and 7.0 Hz, total 3H), 1.03 (d, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 171.3, 170.1, 163.8, 163.6, 147.8, 147.6, 92.4, 79.4(3), 79.3(6), 60.1, 59.2, 59.1, 57.5, 57.3, 51.2, 41.8, 38.2, 38.0, 31.2, 28.1, 27.4, 27.2, 19.0, 17.8, 16.7(2), 16.6(6); IR (KBr) 3608, 3582, 3400, 2964, 1737, 1612, 1462, 1355, 1275, 1150, 903, 856 cm^{-1}; ESMS (−ve) m/z 430 ($M-H^+$); LSIMS-HRMS m/z 432.1803. calcd for $C_{18}H_{30}N_3O_7S$ ($M+H^+$) m/z 432.1804.

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