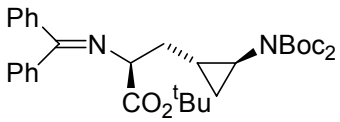
Supplementary information

Total synthesis of (+)-Belactosin A

Alan Armstrong* and James N. Scutt

All compounds **4-7** were prepared as previously described in reference 5b, from their enantiomeric precursors (i.e. **5** synthesised from (*R*)-glycidol benzyl ether etc).

(2*S*, 1'*R*, 2'*S*)- (*N*-(bis-boc)-*N*-(diphenylmethylene)-3-(2-aminocyclopropyl)) alanine *tert*-butyl ester

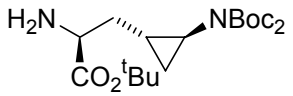


8

Prepared using *O*(9)-allyl-*N*-(9-anthracenylmethyl)-cinchonidinium bromide catalyst, as described in ref 3b. Aminocyclopropyl alanine **8** (67% yield) obtained as colourless needles (m.p. 127-128 °C) after recrystallisation from ether/hexane.

$[\alpha]^{27}_{D}$ -24 (*c* 1.32, $CHCl_3$); ν_{max}/cm^{-1}(film) 3059, 2978, 1787, 1735, 1368, 1285, 1258, 1160, 1116, 801, 783, 698; δ_H (500 MHz, d^8-tol) 7.82-7.80 (2H, m, Ar*H*), 7.16-6.98 (8H, m, Ar*H*), 4.28 (1H, app t, *J*6.3, $Ph_2C{=}NCH$), 2.73-2.69 (1H, m, $Ph_2C{=}NCHCH_2$), 2.41-2.39 (1H, m, $CHNBoc_2$), 1.47-1.28 (29H, m, $Ph_2C{=}NCHCH_2$, Boc_2, $CO_2{}^tBu$, $CH(CH_2)CHNBoc_2$), 0.85-0.81 (1H, m, $(CH_2)CHNBoc_2$), 0.74 (1H, app q, *J*6.5, $(CH_2)CHNBoc_2$); δ_C (125 MHz, $CDCl_3$) 171.0 (C), 169.7 (C), 153.0 (C), 139.6 (C), 136.7 (C), 132.4 (CH), 130.1 (CH), 130.0 (CH), 128.8 (CH), 128.5 (CH), 128.3 (CH), 128.0 (CH), 127.8 (CH), 82.1 (C), 80.9 (C), 65.0 (CH), 36.2 (CH_2), 33.8 (CH), 28.1 (CH_3), 28.0 (CH_3), 20.4 (CH), 16.7 (CH_2); m/z (CI, NH_3) 565 $[M+H]^+$, found : $[M+H]^+$, 565.3304. $C_{33}H_{45}N_2O_6$ requires $[M+H]^+$, 565.3278.

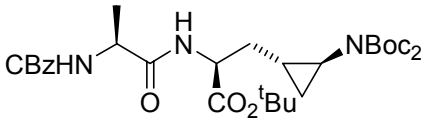
(S)-2-Amino-3-((1S,2S)-2-di-(*tert*-butoxycarbonyl)amino-cyclopropyl)-propionic acid *tert*-butyl ester



9

To a solution of **8** (0.29 g, 0.52 mmol), in THF (5.5 ml), was added aqueous citric acid (2.70 ml, 15 % solution) dropwise at room temperature. The mixture was then stirred rapidly for 1.5 hours, followed by dilution with ethyl acetate (100 ml), washing with saturated sodium hydrogen carbonate (100 ml), then further extraction with ethyl acetate (100 ml). Organic fractions were combined and dried over magnesium sulfate, followed by removal of solvents *in vacuo*. The crude mixture was then purified by column chromatography (ethyl acetate eluent), to afford the desired amine **9** (0.173 g, 84 %) as a clear oil; $[\alpha]^{23}_{D}$ 26.8 (*c* 1.34, $CHCl_3$); ν_{max}/cm^{-1}(film) 3403, 2979, 2933, 1785, 1733, 1367, 1282, 1254, 1159, 1118, 852, 752; δ_H (250 MHz, $CDCl_3$) 3.54 (1H, dd, *J* 7.3, 5.2, $H_2NCH(CO_2{}^tBu)$), 2.41-2.35 (1H, m, $CHNBoc_2$), 1.92-1.80 (2H, m, NH_2, $(CO_2{}^tBu)CHCH_2$)), 1.47-1.28 (29H, m, Boc_2, $CO_2{}^tBu$, $(CO_2{}^tBu)CHCH_2$)), 1.13-1.00 (1H, m, $CH(CH_2)CHNBoc_2$), 0.85-0.81 (2H, m, $(CH_2)CHNBoc_2$); δ_C (100 MHz, $CDCl_3$) 174.5 (C), 153.2 (C), 82.3 (C), 80.9 (C), 54.6 (CH), 37.6 (CH_2), 33.9 (CH), 28.0 (CH_3), 19.4 (CH), 16.7 (CH_2); m/z (CI, NH_3) 401 $[M+H]^+$, found : $[M+H]^+$, 401.2654. $C_{20}H_{37}N_2O_6$ requires $[M+H]^+$, 401.2652.

***N*-CBz-L-alanine-(S)-2-Amino-3-((1S,2S)-2-di-(*tert*-butoxycarbonyl)amino-cyclopropyl)-propionic acid *tert*-butyl ester**

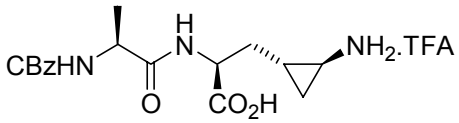


10

To a mixture of DCC (33 mg, 0.16 mmol) and HOBt (43 mg, 0.32 mmol) in DMF (1 ml), was added a solution of amine **9** (32 mg, 0.08 mmol) and *N*-CBz-Ala (36 mg,

0.16 mmol) in DMF (1 ml), followed by further washing with DMF (0.5 ml). The mixture was then stirred at room temperature for 1 hour. DMF was removed at high vacuum (warm water bath), followed by addition of ether (3 ml) and filtration of the urea by-product. The filtrate was then concentrated and purified by column chromatography (2 ether : 1 petrol eluent) to afford the desired amino acid **10** (50 mg, 100 %) as a white foam; $[\alpha]^{20}_{D}$ 6.0 (*c* 2.0, $CHCl_3$); ν_{max}/cm^{-1}(film) 3314, 2980, 2933, 1726, 1674, 1368, 1274, 1257, 1160, 1121, 738; δ_H (250 MHz, $CDCl_3$) 8.75 (1H, d, *J* 9.5, N*H*CH($CO_2{}^tBu$)), 7.36-7.28 (5H, m, Ar), 5.73 (1H, d, *J* 7.3, CBzN*H*), 5.10 (2H, s, $PhCH_2$), 4.77-4.70 (1H, m, NHC*H*($CO_2{}^tBu$)), 4.35 (1H, app quintet, *J* 7.3, CBzNHC*H*(Me)), 2.48-2.40 (2H, m, C*H*$NBoc_2$, NHCH($CO_2{}^tBu$)CH_2), 1.51 (18H, s, Boc_2), 1.44 (9H, s, $CO_2{}^tBu$), 1.18-1.05 (1H, m, NHCH($CO_2{}^tBu$)CH_2), 0.98-0.84 (1H, m, C*H*(CH_2)$CHNBoc_2$), 0.80-0.72 (1H, m, (CH_2)$CHNBoc_2$), 0.66 (1H, app q, (CH_2)$CHNBoc_2$); δ_C (100 MHz, $CDCl_3$) 171.9 (C), 170.3 (C), 155.6 (C), 153.8 (C), 136.5 (C), 128.5 (CH), 128.0 (CH), 128.0 (CH), 83.2 (C), 81.7 (C), 66.6 (CH_2), 51.5 (CH), 50.2 (CH), 35.1 (CH), 34.4 (CH_2), 28.0 (CH_3), 19.8 (CH), 16.7 (CH_2), 13.7 (CH_3); m/z (CI, NH_3) 606 $[M+H]^+$, found : $[M+H]^+$, 606.3381. $C_{31}H_{48}N_3O_9$ requires $[M+H]^+$, 606.3391.

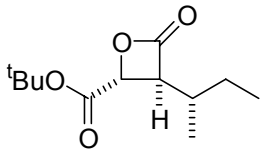
_N_-CBz-L-alanine-(S)-2-Amino-3-((1R,2S)-2-amino-cyclopropyl)-propionic acid . TFA



11

To a stirred solution of amino acid **10** (200 mg, 0.33 mmol) in dichloromethane (3.70 ml) at 0 °C was added TFA (3.70 ml) dropwise, and the mixture was placed in a fridge (approx 15 ºC) for 20 h. Solvents were removed *in vacuo*, and the mixture was diluted with distilled water (25 ml), and washed with ether (25 ml). Aqueous fractions were concentrated *in vacuo*, and the residue was then freeze dried to afford the desired amine salt **11** (0.137 g, 90 %). This material was used directly in the next step without further purification.

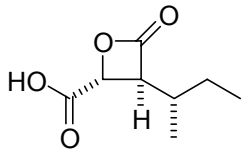
(2R,3S)-3-((S)-*sec*-Butyl)-4-oxo-oxetane-2-carboxylic acid *tert*-butyl ester



19

To a cooled solution of acid **18** (0.69 g, 2.71 mmol), in THF (20 ml) at –78 ˚C, was added LiHMDS (6.54 ml, 6.54 mmol, 1.00 M solution in hexanes), dropwise. After stirring at –78 °C for 45 min carbon tetrachloride (0.316 ml, 3.268 mmol) was added dropwise. The mixture was then stirred at –78 °C for 30 min, then allowed to warm to room temperature and stir for a further 10 min. Most of the solvent was removed *in vacuo* followed by addition of ether (20 ml) then 5 % aqueous sodium bicarbonate (20 ml) with subsequent rapid stirring for 20 h. The mixture was diluted with ether (150 ml), washed with saturated sodium bicarbonate (2 x 50 ml), then dried over magnesium sulfate and concentrated *in vacuo*. The crude material was purified by flash column chromatography (3 petrol : 1 ether eluent), affording the desired β-lactone **19** (0.38 g, 55 %) as a white solid, which was recrystallised (ether/petrol) to afford **19** as white needles (m.p. 44-45 °C); $[\alpha]^{20}_{D}$ –8.2 (*c* 0.73, $CHCl_3$); ν_{max}/cm^{-1}(film) 2966, 2934, 2879, 1839, 1752, 1703, 1460, 1370, 1238, 1157, 1105, 1010, 930; δ_H (250 MHz, $CDCl_3$) 4.53 (1H, d, *J* 4.3, $^tBuO_2CCH$), 3.58 (1H, dd, *J* 7.8, 4.3, $^tBuO_2CCHCH$), 2.05-1.88 (1H, m, $CH(CH_3)Et$), 1.68-1.51 (1H, m, CH_2CH_3), 1.48 (9H, s, $CO_2{}^tBu$), 1.39-1.21 (1H, m, CH_2CH_3), 1.01 (1H, d, *J* 6.7, $CH(CH_3)Et$), 0.91 (1H, app t, J 7.6, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 11.0 (CH_3), 16.3 (CH_3), 26.8 (CH_2), 27.9 (CH_3), 33.5 (CH_2), 62.4 (CH), 69.5 (CH), 83.5 (C), 167.5 (C), 169.1 (C); m/z (CI, NH_3) 246 $[M+NH_4]^+$, found : $[M+NH_4]^+$, 246.1696. $C_{12}H_{24}NO_4$ requires $[M+NH_4]^+$, 246.1705.

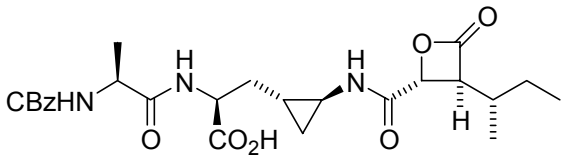
(2R,3S)-3-((S)-*sec*-Butyl)-4-oxo-oxetane-2-carboxylic acid



3

To a cooled solution of β-lactone **19** (0.14 g, 0.59 mmol) in dichloromethane (4 ml) at 0 °C, was added TFA dropwise (4 ml). The mixture was then stirred at this temperature for 15 hours, after which solvents were removed *in vacuo*, and the residue azeotroped from toluene (4 ml). After further concentration the mixture was then purified by column chromatography (9 $CHCl_3$: 1 MeOH : 0.1 AcOH eluent), to afford acid **3** (0.92 g, 90 %) as a clear oil. This material was used directly in the next step.

***N*-CBz belactosin A**



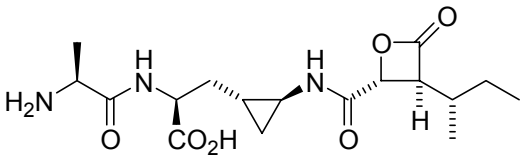
20

To a cooled solution of β-lactone **3** (36 mg, 0.21 mmol) in CH_2Cl_2 (2.30 ml) at 0 ˚C was added distilled water (2.30 ml), then HOBt (0.114 g, 0.84 mmol) and EDCI (81 mg, 0.42 mmol). The biphasic mixture was then stirred rapidly at 0 ˚C for 10 min, followed by transfer of the organic phase dropwise to a cooled solution (0 ˚C) of amine salt **11** (65 mg, 0.14 mmol) and Hunig's base (73 μl, 0.42 mmol) in DMF (1 ml) (previously stirred for 10 min). This mixture was then further stirred at this temperature for 1 hour, followed by removal of all solvents at high vacuum. The crude material was directly purified by column chromatography (9.5 $CHCl_3$: 0.5 MeOH : 0.1 AcOH eluent), then recrystallised from ethyl acetate/pentane to afford *N*-CBz belactosin A **20** (35 mg, 50 %) as a white foam; $[\alpha]^{23}_D$ –8.7 (*c* 0.69, $CHCl_3$);

ν_{max}/cm^{-1}(film) 3416, 2964, 1837, 1717, 1662, 1525, 1454, 1260, 1097, 909, 733; δ_H (400 MHz, d_6-acetone) 9.03 (1H, d, *J* 9.1, N*H*CH(CO_2H)), 8.02 (1H, s, (CH_2)CHN*H*C(O)), 7.38-7.27 (5H, m, Ar), 6.41 (1H, d, *J* 7.7, CBzN*H*), 5.06 (2H, s, $PhCH_2$), 4.81 (1H, d, *J* 4.4, NHC(O)C*H*), 4.78-4.74 (1H, m, NHC*H*(CO_2H)), 4.43-4.36 (1H, m, CBzNHC*H*(Me)), 3.70 (1H, dd, *J* 8.0, 4.4, NHC(O)CHC*H*), 2.64-2.62 (1H, m, C*H*NHC(O)), 2.36 (1H, app dt, *J* 14.6, 2.9, NHCH(CO_2H)CH_2), 1.98-1.92 (1H, m, C*H*(CH_3)Et), 1.69-1.59 (1H, m, CH_2CH_3), 1.39 (3H, d, *J* 7.0, CBzNHCH(CH_3)), 1.32-1.18 (2H, m, NHCH(CO_2H)CH_2, CH_2CH_3), 1.03 (1H, d, *J* 6.6, CH(CH_3)Et), 0.96-0.81 (5H, m, CH_2CH_3, C*H*(CH_2)CHNH, (CH_2)CHNH), 0.61-0.56 (1H, m, (CH_2)CHNH); δ_C (100 MHz, $CDCl_3$) 174.3 (C), 173.8 (C), 171.1 (C), 168.7 (C), 155.9 (C), 136.2 (C), 128.5 (CH), 128.1 (CH), 127.9 (CH), 70.2 (CH), 66.7 (CH_2), 62.9 (CH), 51.5 (CH), 50.4 (CH), 33.8 (CH), 33.4 (CH_2), 29.5 (CH), 26.7 (CH_2), 19.1 (CH_3), 16.8 (CH), 16.3 (CH_3), 11.0 (CH_3), 10.2 (CH_2); m/z (FAB, +ve) 504 $[M+H]^+$, found : $[M+H]^+$, 504.2345. $C_{25}H_{34}N_3O_8$ requires $[M+H]^+$, 504.2346.

Belactosin A [2a]

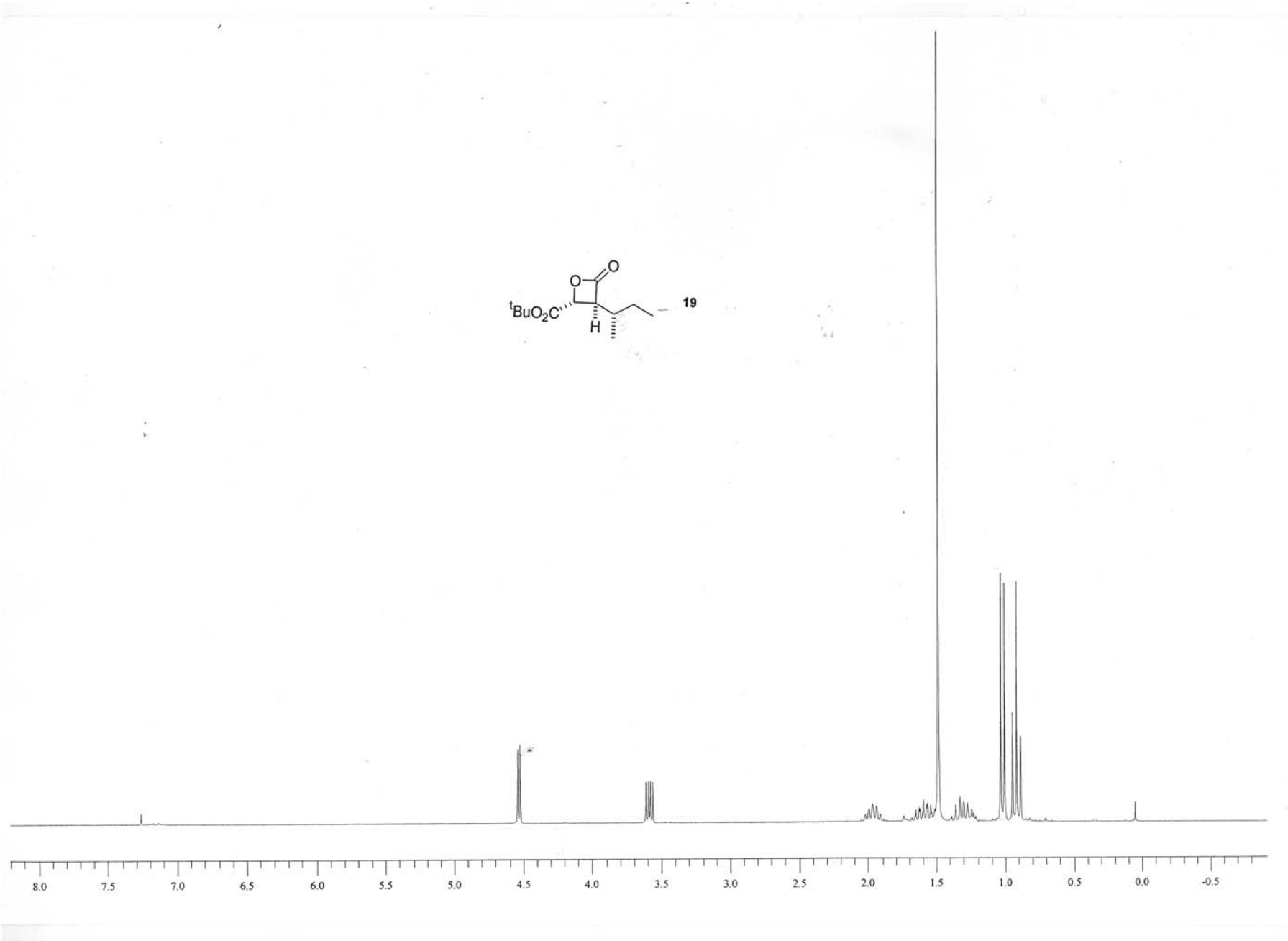
[2a] T. Mizukami, A. Asai, Y. Yamashita, R. Katahira, A. Hasegawa, K. Ochiai and S. Akinaga, Eur. Patent 768317, 1997.



1

To a mixture of *N*-CBz belactosin A **20** (24 mg, 0.048 mmol) and Pd/C (24 mg), was added THF (1ml) and the mixture was thoroughly purged with H_2 until the solvent volume was approximately 0.30 ml. To this suspension was added formic acid (0.20 ml), and the mixture was placed under a balloon pressure of H_2, and stirred at room temperature for 2.5 hours. The suspension was then filtered, washed with CH_2Cl_2 (2 x 2 ml), and concentrated *in vacuo*. Distilled water was added (2 x 1 ml), and the dissolved filtrate was again filtered then concentrated at high vacuum. The residue

was finally azeotroped with toluene/MeOH (2:1, 3 ml), to afford pure belactosin A **1** (17 mg, 96 %) as a white solid (m.p. 186-187 ˚C); $[\alpha]^{21}_{D}$ +4.8 (*c* 0.84, H_2O); ν_{max}/cm^{-1}(film) 3252, 3074, 2964, 1832, 1674, 1598, 1397, 1205, 1139, 911, 801, 723; δ_H (500 MHz, D_2O) 4.92 (1H, d, *J* 4.5, NHC(O)C*H*), 4.40 (1H, app t, *J* 5.7, NHC*H*(CO_2H)), 4.20 (1H, q, *J* 7.1, H_2NC*H*(Me)), 3.90 (1H, dd, *J* 7.4, 4.5, NHC(O)CHC*H*), 2.58 (1H, app dt, *J* 7.4, 3.7, C*H*NHC(O)), 2.11-2.05 (1H, m, C*H*(CH_3)Et), 1.96 (1H, app dt, *J* 14.4, 6.0, NHCH(CO_2H)CH_2), 1.70 (1H, ddd, *J* 14.4, 8.4, 5.6, NHCH(CO_2H)CH_2), 1.63 (3H, d, *J* 7.1, H_2NCH(CH_3)), 1.62-1.55 (1H, m, CH_2CH_3), 1.41-1.32 (1H, m, CH_2CH_3), 1.06 (1H, d, *J* 6.7, CH(CH_3)Et), 1.02-0.98 (1H, m, C*H*(CH_2)CHNH), 0.95 (1H, app t, *J* 7.4, CH_2CH_3), 0.93-0.87 (1H, m, (CH_2)CHNH), 0.77 (1H, app dt, *J* 7.4, 6.0, (CH_2)CHNH); δ_C (62 MHz, D_2O) 178.5 (C), 173.5 (C), 172.7 (C), 170.8 (C), 71.7 (CH), 62.7 (CH), 55.7 (CH), 50.0 (CH), 34.5 (CH_2), 33.6 (CH), 29.2 (CH), 27.0 (CH_2), 17.3 (CH_3), 16.7 (CH), 16.2 (CH_3), 12.0 (CH_2), 11.1 (CH_3); m/z (FAB, +ve) 370 $[M+H]^+$, found : $[M+H]^+$, 370.1981. $C_{25}H_{34}N_3O_8$ requires $[M+H]^+$, 370.1978.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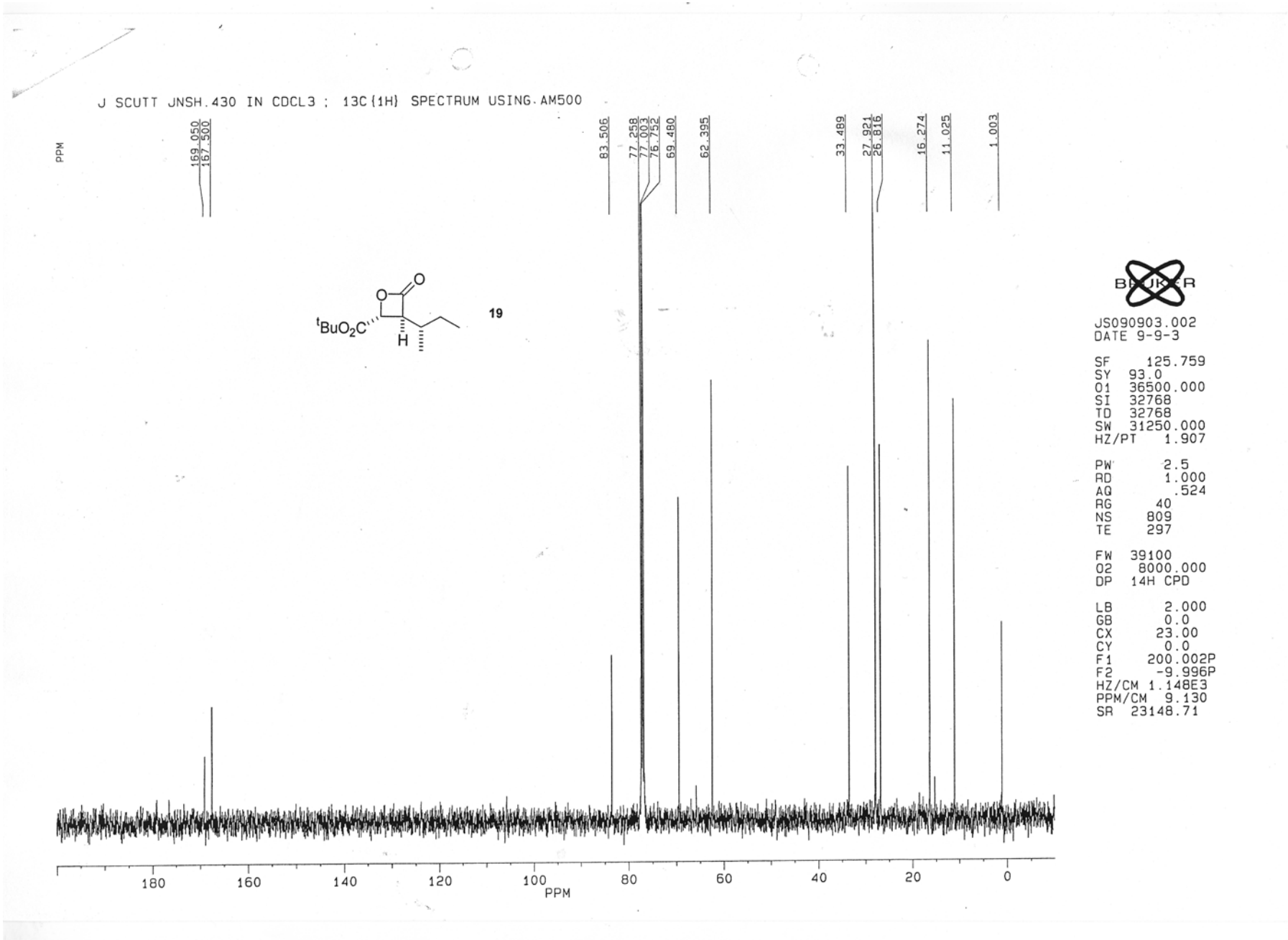
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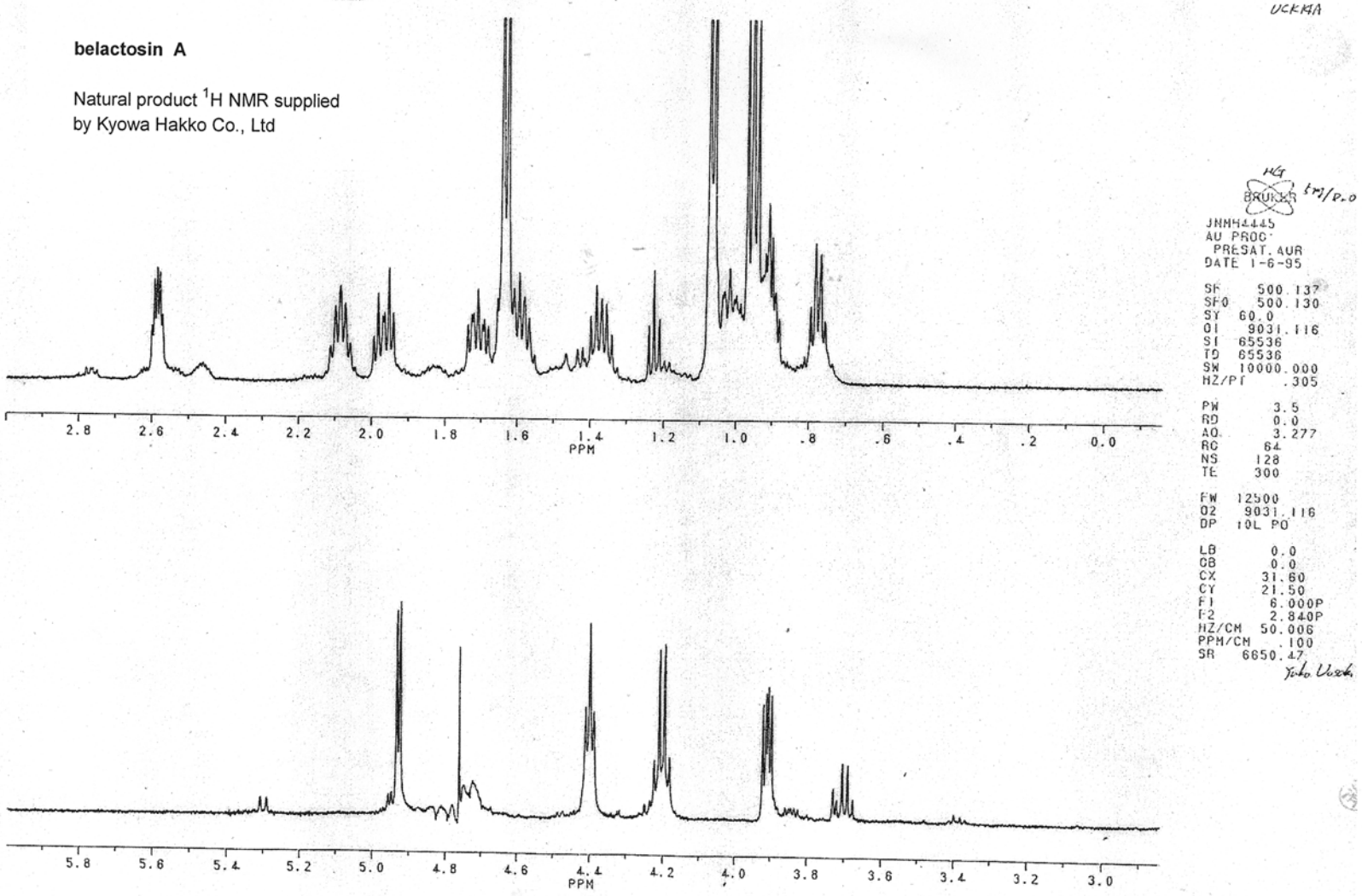
belactosin A

Natural product ^{1}H NMR supplied
by Kyowa Hakko Co., Ltd



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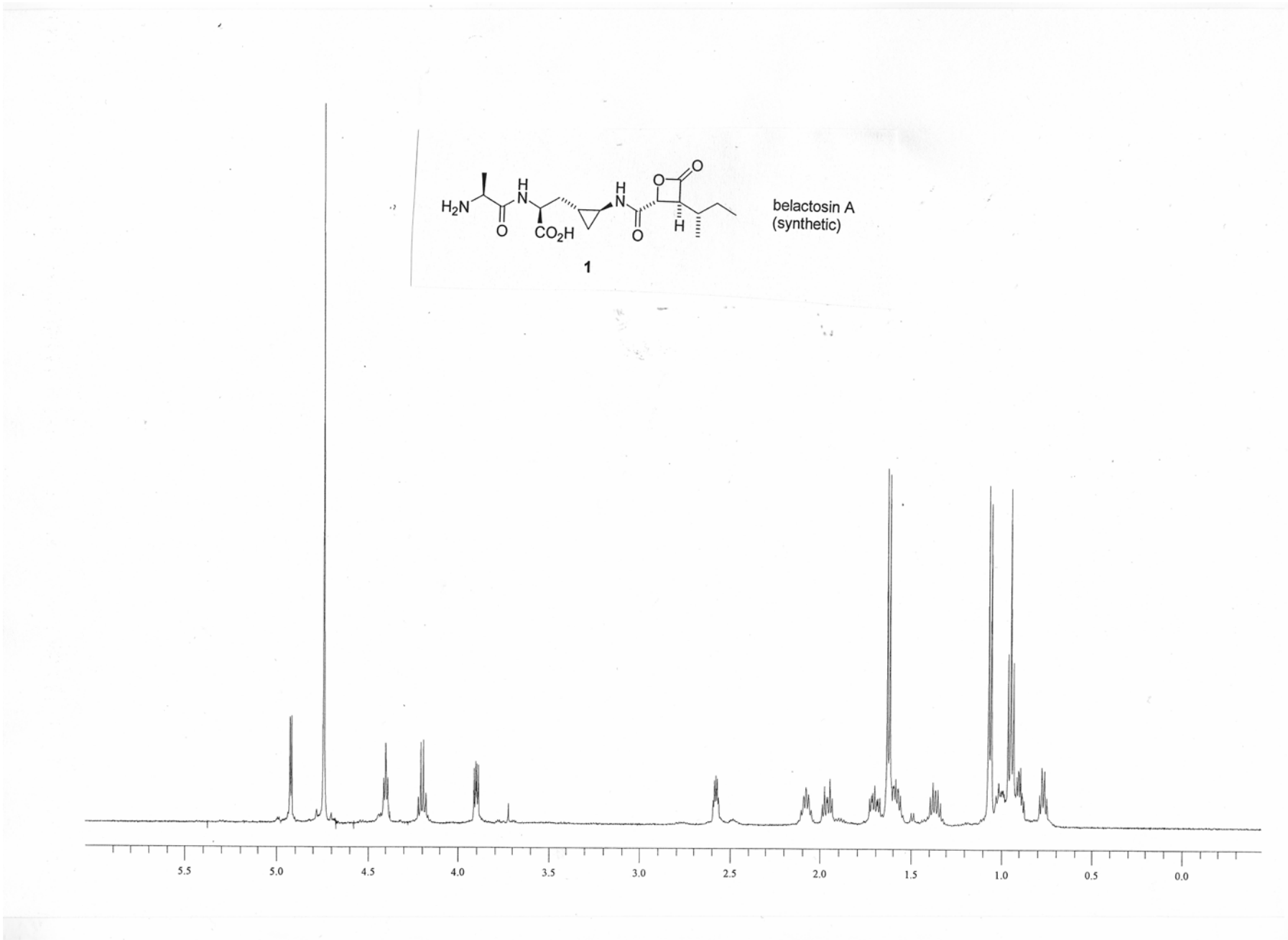
Yoko Usui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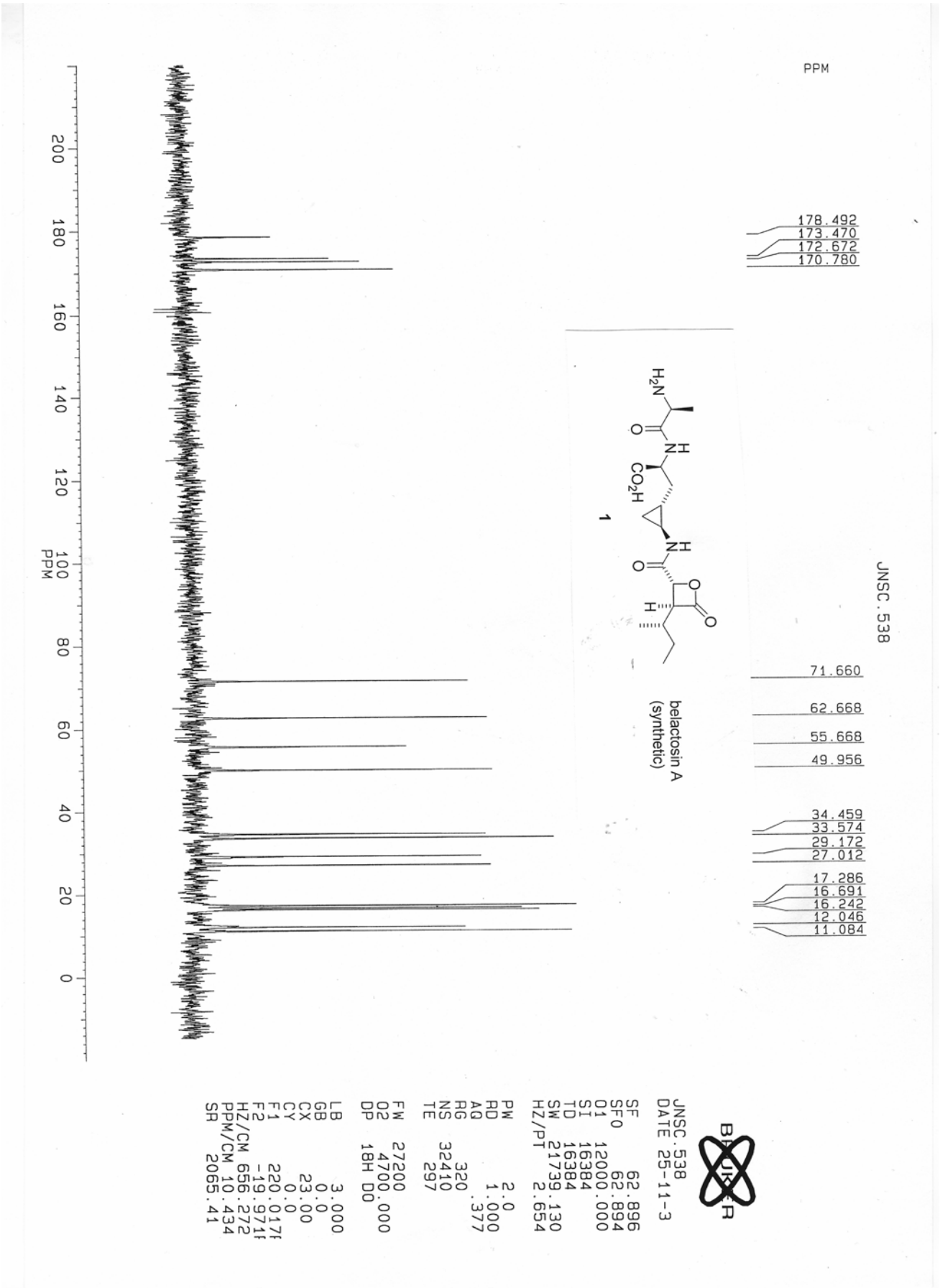
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X-ray data for compound 19

Table 1. Crystal data and structure refinement for AA0305.

Identification code	AA0305
Empirical formula	C12 H20 O4
Formula weight	228.28
Temperature	293(2) K
Diffractometer, wavelength	Bruker P4, 1.54178 Å
Crystal system, space group	Orthorhombic, P2(1)2(1)2(1)
Unit cell dimensions	a = 6.0476(7) Å α = 90°
	b = 11.4445(7) Å β = 90°
	c = 19.263(3) Å γ = 90°
Volume, Z	1333.2(3) $Å^3$, 4
Density (calculated)	1.137 Mg/m^3
Absorption coefficient	0.692 mm^{-1}
F(000)	496
Crystal colour / morphology	Colourless needles
Crystal size	1.00 x 0.33 x 0.10 mm^3
θ range for data collection	4.59 to 64.99°
Index ranges	0<=h<=7, 0<=k<=13, -22<=l<=0
Reflns collected / unique	1342 / 1342 [R(int) = 0.0000]
Reflns observed [F>4σ(F)]	1091
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1342 / 0 / 146
Goodness-of-fit on F^2	1.059
Final R indices [F>4σ(F)]	R1 = 0.0509, wR2 = 0.1389
	R1+ = 0.0509, wR2+ = 0.1389
	R1- = 0.0511, wR2- = 0.1395
R indices (all data)	R1 = 0.0617, wR2 = 0.1479
Absolute structure parameter	x+ = 0.0(6), x- = ******
Extinction coefficient	0.033(3)
Largest diff. peak, hole	0.144, -0.124 $eÅ^{-3}$
Mean and maximum shift/error	0.000 and 0.000

Table 2. Bond lengths [Å] and angles [°] for AA0305.

O(1)-C(2)	1.343(6)
O(1)-C(4)	1.461(4)
C(2)-O(2)	1.189(6)
C(2)-C(3)	1.513(5)
C(3)-C(5)	1.518(5)
C(3)-C(4)	1.538(5)
C(4)-C(9)	1.522(5)
C(5)-C(8)	1.527(5)
C(5)-C(6)	1.532(6)
C(6)-C(7)	1.515(6)
C(9)-O(9)	1.187(4)
C(9)-O(10)	1.328(4)
O(10)-C(11)	1.484(4)
C(11)-C(12)	1.498(6)
C(11)-C(14)	1.511(6)
C(11)-C(13)	1.518(5)

C(2)-O(1)-C(4)	91.6(3)
O(2)-C(2)-O(1)	126.8(4)
O(2)-C(2)-C(3)	137.5(5)
O(1)-C(2)-C(3)	95.8(3)
C(2)-C(3)-C(5)	119.3(3)
C(2)-C(3)-C(4)	82.5(3)
C(5)-C(3)-C(4)	120.0(3)
O(1)-C(4)-C(9)	111.3(3)
O(1)-C(4)-C(3)	90.0(3)
C(9)-C(4)-C(3)	114.1(3)
C(3)-C(5)-C(8)	109.2(3)
C(3)-C(5)-C(6)	109.1(3)
C(8)-C(5)-C(6)	112.8(3)
C(7)-C(6)-C(5)	114.2(4)
O(9)-C(9)-O(10)	127.6(4)
O(9)-C(9)-C(4)	123.5(3)
O(10)-C(9)-C(4)	108.8(3)
C(9)-O(10)-C(11)	121.9(3)
O(10)-C(11)-C(12)	109.7(3)
O(10)-C(11)-C(14)	108.6(3)
C(12)-C(11)-C(14)	113.0(4)
O(10)-C(11)-C(13)	103.0(3)
C(12)-C(11)-C(13)	110.7(4)
C(14)-C(11)-C(13)	111.4(4)

Symmetry transformations used to generate equivalent atoms: