Electronic Supplementary Material (ESI) for Chemical Communications

α-L-ARABINOFURANOSYLATED PYRROLIDINES AS ARABINANASE INHIBITORS

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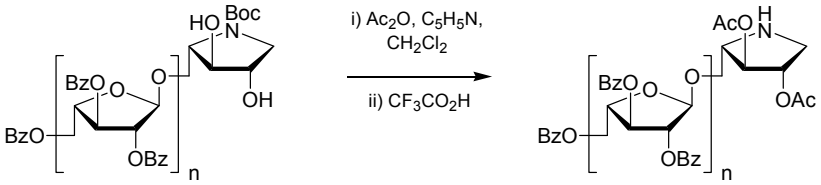
SUPPLEMENTARY MATERIAL

Structural Assignment of Compounds (7) and (8) by Chemical Modification

Compound **7** was acetylated by the action of acetic anhydride and pyridine in dichloromethane. The product was treated with anhydrous trifluoroacetic acid to return, after basic workup, the amine **S1**. The ^{1}H and ^{13}C NMR spectra of the amine **S1** confirmed that the product was of α-configuration; H-1′ appeared as an apparent singlet at 5.31 ppm in the ^{1}H NMR spectrum, whilst in the ^{13}C NMR spectrum the C-1 resonance appeared at 106.0 ppm.[1,2] The (1→5) nature of the glycosidic linkage was confirmed by the downfield chemical shift of H-2 and H-3 at 5.53 and 5.60 ppm, respectively.

Similarly, compound **8** was acetylated then treated with anhydrous trifluoroacetic acid to provide, after basic workup, the amine **S2**. The ^{1}H and ^{13}C NMR spectra of the amine **S2** were commensurate with the assigned structure.[1,2]



7 n = 1
8 n = 2

S1 n = 1 (78 %)
S2 n = 2 (84 %)

References

(1) K. Mizutani, R. Kasai, M. Nakamura, O. Tanaka, H. Matsuura, *Carbohydr. Res.* 1989, **185**, 27.

(2) M. Joe, Y. Bai, R. C. Nacario, T. L. Lowary, *J. Am. Chem. Soc.* 2007, **129**, 9885.

Experimental

General Experimental Procedures

Unless stated otherwise, ^{1}H NMR spectra were calibrated using the residual solvent peak and ^{13}C NMR spectra were calibrated using the most prominent solvent ^{13}C resonance.[1] NMR spectra run in D_2O used internal MeOH [^{1}H, δ = 3.34 (CH_3) and ^{13}C, δ = 49.0] as the standard.[1] Peak assignments in NMR spectra were made, where possible, with the assistance of COSY-45 and HSQC experiments.

Melting points were determined on a hot stage melting point apparatus. Optical rotations were performed in a microcell (1.0 ml, 10 cm path length) at a concentration of 10 mg.ml^{-1} in $CHCl_3$, unless otherwise stated, at room temperature. Mass spectra were recorded with the fast atom bombardment (FAB) technique, with 3-nitrobenzyl alcohol as a matrix, unless otherwise stated.

Flash chromatography was performed on BDH silica gel or Geduran silica gel 60 with the specified solvents. Thin layer chromatography (t.l.c.) was effected on Merck silica gel 60 F_{254} aluminium-backed plates that were stained by heating (> 200 °C) with 5% H_2SO_4 in EtOH.

Percentage yields for chemical reactions as described are quoted only for those compounds that were purified by recrystallisation or by column chromatography and the purity assessed by ^{1}H NMR spectroscopy.

All solvents were distilled prior to use and dried according to the methods of Burfield.[2]

'Standard workup' refers to dilution with water, repeated extraction into an organic solvent, sequential washing of the combined organic extracts with HCl (1 M, where appropriate), sat. aq. $NaHCO_3$ and NaCl solutions, followed by drying over anhydrous $MgSO_4$, filtration, and evaporation of the solvent by means of a rotary evaporator at reduced pressure.

References

(1) Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* 1997, **62**, 7512.

(2) Burfield, D. R.; Smithers, R. H.; *J. Org. Chem.* 1983, **48**, 2420.

Glycosylation procedure

A mixture of the trichloroacetimidate (1.1 mmol), the alcohol (1.0 mmol) and crushed activated molecular sieves (4 Å, 1.0 g) in dry CH_2Cl_2 (25 ml) was stirred under an atmosphere of Ar (30 min) and cooled (−40°C). Trimethylsilyl trifluoromethanesulfonate (0.10 ml) was added and the mixture stirred (0 °C, 1 h). Triethylamine (0.50 ml) was added and the mixture filtered through Celite and washed with CH_2Cl_2 (3 × 10 ml). The combined filtrate/washings were concentrated and flash chromatography gave the glycoside.

(2S,3S,4S)-1-(*tert*-Butoxycarbonyl)-3,4-dihydroxy-2-(hydroxymethyl)pyrrolidine (4)

Di-*tert*-butyl dicarbonate (2.0 g, 9.0 mmol) was added to the amine **3**[19] (1.0 g, 6.0 mmol) in MeOH/Et_3N (3:1, 20 ml) and the solution kept at room temperature (4 h). The mixture was concentrated and flash chromatography (MeOH:EtOAc, 1:19) gave the carbamate **4** as colourless needles (1.2 g, 87%), m.p. 117–118 ˚C (EtOAc), $[\alpha]_D$ +17.0 (MeOH). ^{1}H NMR [600 MHz, $(CD_3)_2CO$] δ = 1.44 [s, 9 H, $C(CH_3)_3$], 3.24–3.31 (bm, 1 H), 3.64–3.75 (bm, 3 H), 3.86–4.18 (bm, 3 H), 4.31–4.92 (bm, 3 H). ^{13}C NMR [150.9 MHz, $(CD_3)_2CO$] δ = 28.6 [$C(CH_3)_3$], 54.7 (CH_2OH), 62.1, 62.8 (C-5), 68.1, 68.4, 75.4, 75.9, 79.2, 80.1 (C-2,3,4), 79.4, 79.7 [$C(CH_3)_3$], 155.2, 156.3 (C=O). HRMS (FAB): m/z = 234.1346; $[M + H]^+$ requires 234.1341. Anal. Calcd. for $C_{10}H_{19}NO_5$: C, 51.5; H, 8.2; N, 6.0. Found: C, 51.6; H, 8.2; N, 5.7.

Benzyl 2,3,5-Tri-*O*-benzoyl-*α*-L-arabinoside

The trichloroacetimidate **5**[18] (2.4 g, 4.0 mmol) and BnOH (0.46 ml, 4.4 mmol) were subjected to the general glycosylation procedure [flash chromatography (EtOAc/petrol, 1:9)] to give the title compound as a colourless oil (1.9 g, 88%), $[\alpha]_D$ −10.8. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 4.63 (ddd, 1 H, *J* = 3.6, 4.6, 5.1 Hz, H-4), 4.68, 4.90 (ABq, 2 H, *J* = 12.0 Hz, CH_2Ph), 4.71 (dd, 1 H, *J* = 5.1, 11.9 Hz, H-5), 4.85 (dd, 1 H, *J* = 3.6, 11.9 Hz, H-5), 5.42 (s, 1 H, H-1), 5.62–5.64 (m, 2 H, H-2,3), 7.30–8.09 (m, 20 H, Ph); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ = 63.9 (C-5), 69.0 (CH_2Ph), 78.0 (C-4), 81.5, 82.1 (C-2,3), 105.1 (C-1), 127.9–137.4 (Ph), 165.5, 165.9, 166.3 (C=O). HRMS (FAB): m/z = 553.1820; $[M + H]^+$ requires 553.1862. Anal. Calcd. for $C_{33}H_{28}O_8$: C, 71.7; H, 5.1. Found: C, 71.8; H, 5.2.

Benzyl *α*-L-Arabinofuranoside

Sodium (10 mg) was added to benzyl 2,3,5-tri-*O*-benzoyl-*α*-L-arabinoside (1.7 g) in MeOH (20 ml) and the resulting solution stirred (2 h). The solution was neutralised (Amberlite IR-120, H^+), filtered and

concentrated. Flash chromatography (EtOAc/petrol, 9:1) gave the title compound as a colourless oil (0.66 g, 91%), $[\alpha]_D$ –109 (MeOH). ^{1}H NMR [500 MHz, $(CD_3)_2SO$] δ = 3.44 (ddd, 1 H, J = 5.6, 5.9, 11.8 Hz, H-5), 3.58 (ddd, 1 H, J = 3.3, 4.6, 11.8 Hz, H-5), 3.66 (ddd, 1 H, J = 4.7, 4.8, 7.1 Hz, H-3), 3.79 (ddd, 1 H, J = 3.3, 5.6, 7.1 Hz, H-4), 3.85 (ddd, 1 H, J = 1.9, 5.3, 4.7 Hz, H-2), 4.44, 4.65 (ABq, 2 H, J = 12.1 Hz, CH_2Ph), 4.73 (bdd, 1 H, J = 4.6, 5.9 Hz, OH), 4.82 (d, 1 H, J = 1.9 Hz, H-1), 5.12 (bd, 1 H, J = 5.3 Hz, OH), 5.32 (bd, 1 H, J = 4.8 Hz, OH), 7.26–7.38 (m, 5 H, Ph); ^{13}C NMR [125.8 MHz, $(CD_3)_2SO$] δ = 61.8 (C-5), 68.6 (*C*H$_2$Ph), 77.6, 82.6, 84.5 (C-2,3,4), 107.7 (C-1), 127.8, 128.1, 128.6, 138.6 (Ph). HRMS (FAB): m/z = 241.1070; $[M + H]^+$ requires 241.1076. Anal. Calcd. for $C_{12}H_{16}O_5$: C, 60.0; H, 6.7. Found: C, 59.7; H, 6.7.

Benzyl Tri-*O*-benzoyl-*α*-L-arabinofuranosyl-(1→5)-*α*-L-arabinofuranoside

The trichloroacetimidate **5**[18] (1.33 g, 2.20 mmol) and benzyl *α*-L-arabinofuranoside (480 mg, 2.00 mmol) were subjected to the general glycosylation procedure [flash chromatography (EtOAc/petrol, 3:7)] to give the title compound as a colourless oil (1.04 g, 76%), $[\alpha]_D$ –37.2. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.83 (bd, 1 H, J = 9.2 Hz, OH), 3.33 (bd, 1 H, J = 10.7 Hz, OH), 3.85 (dd, 1 H, J = 2.6, 11.0 Hz, H-5), 4.06–4.13 (m, 3 H, H-2,3,5), 4.27 (ddd, 1 H, J = 2.2, 2.2, 2.6 Hz, H-4), 4.55, 4.76 (ABq, 2 H, J = 10.7 Hz, CH_2Ph), 4.59 (ddd, 1 H, J = 3.3, 4.9, 4.9 Hz, H-4′), 4.69 (dd, 1 H, J = 4.9, 12.0 Hz, H-5′), 4.86 (dd, 1 H, J = 3.3, 12.0 Hz, H-5′), 5.13, 5.38 (2 s, 2 H, H-1,1′), 5.50 (d, 1 H, J = 1.3 Hz, H-2′), 5.62 (dd, 1 H, J = 1.3, 4.9 Hz, H-3′), 7.28–8.17 (m, 20 H, Ph); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ = 63.7, 67.1 (C-5,5′), 69.2 (*C*H$_2$Ph), 77.6, 78.3, 79.5, 82.0, 82.1, 86.0 (C-2,3,4,2′,3′,4′), 106.3, 107.3 (C-1,1′), 128.2–137.1 (Ph), 165.6, 166.0, 166.3 (3C, C=O). HRMS (ES): m/z = 707.2151; $[M + Na]^+$ requires 707.2104. Anal. Calcd. for $C_{38}H_{36}O_{12}$: C, 66.7; H, 5.3. Found: C, 66.7; H, 5.1.

Benzyl Tri-*O*-benzoyl-*α*-L-arabinofuranosyl-(1→5)-di-*O*-benzoyl-*α*-L-arabinofuranoside

Benzoyl chloride (0.52 ml, 4.5 mmol) was added to benzyl tri-*O*-benzoyl-*α*-L-arabinofuranosyl-(1→5)-*α*-L-arabinofuranoside (1.0 g, 1.5 mmol) in C_5H_5N (3.0 ml) at 0 ˚C and the mixture stirred (r.t., 3 h). Methanol (0.5 ml) was added and the mixture stirred (10 min). The solvent was removed and a standard workup (EtOAc) followed by flash chromatography (EtOAc/petrol, 1:4) gave the title compound as a colourless oil (1.2 g, 91%), $[\alpha]_D$ –9.5. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 4.00 (dd, 1 H, J = 3.1, 11.2 Hz, H-5), 4.23 (dd, 1 H, J = 4.8, 11.2 Hz, H-5), 4.51 (ddd, 1 H, J = 3.1, 4.8, 4.9 Hz, H-4), 4.62, 4.84 (ABq, 2 H, J = 11.9 Hz, CH_2Ph), 4.67 (dd, 1 H, J = 4.9, 11.8 Hz, H-5′), 4.74 (ddd, 1 H, J =

3.5, 4.9, 4.9 Hz, H-4′), 4.82 (dd, 1 H, *J* = 3.5, 11.8 Hz, H-5′), 5.35, 5.45 (2 s, 2 H, H-1,1′), 5.58–5.66 (m, 4 H, H-2,3,2′,3′), 7.25–8.04 (m, 30 H, Ph); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ = 64.0, 66.7 (C-5,5′), 68.9 (CH_2Ph), 77.7, 78.1, 81.5, 82.1, 82.2, 82.4 (C-2,3,4,2′,3′,4′), 105.2, 106.2 (C-1,1′), 127.9–137.7 (Ph), 165.4, 165.5, 165.87, 165.91, 166.4 (5 C, C=O). HRMS (FAB): m/z = 893.2840; $[M + H]^+$ requires 893.2809. Anal. Calcd. for $C_{52}H_{44}O_{14}$: C, 70.0; H, 5.0. Found: C, 70.1; H, 5.2.

Tri-*O*-benzoyl-*α*-L-arabinofuranosyl-(1→5)-di-*O*-benzoyl-L-arabinofuranosyl Trichloroacetimidate (6)

Palladium on charcoal (50 mg, 10% w/w) was added to benzyl tri-*O*-benzoyl-*α*-L-arabinofuranosyl-(1→5)-di-*O*-benzoyl-*α*-L-arabinofuranoside (1.2 g, 1.3 mmol) in MeOH/EtOAc (1:1, 20 ml) and the mixture stirred under an atmosphere of hydrogen (24 h). The mixture was filtered through Celite, which was washed with EtOAc (3 × 10 ml) and the combined filtrate/washings concentrated. 1,5-Diazabicylo[5.4.0]undec-5-ene (30 µl, 0.20 mmol) was added to a stirred solution of the residue and Cl_3CCN (0.15 ml, 1.5 mmol) in CH_2Cl_2 (15 ml) and the solution kept at room temperature (2 h). The dark solution was concentrated and flash chromatography (EtOAc/petrol, 1:4) gave the presumed trichloroacetimidate **6** as a pale yellow oil (960 mg, 78%) that was used in subsequent glycosylations without further characterization.

(2*S*,3*S*,4*S*)-3,4-Dihydroxy-1-(*tert*-butoxycarbonyl)-2-[(tri-*O*-benzoyl-*α*-L-arabinofuranosyloxy)methyl]pyrrolidine (7)

The trichloroacetimidate **5**[18] (935 mg, 1.54 mmol) and the triol **4** (327 mg, 1.40 mmol) were subjected to the general glycosylation procedure [flash chromatography (EtOAc/PhMe, 1:1)] to give the pseudo-disaccharide **7** as a colourless oil (598 mg, 63%), $[\alpha]_D$ +29.0. The ^{1}H and ^{13}C NMR spectra were uninterpretably broad. HRMS (FAB): m/z = 678.2584; $[M + H]^+$ requires 678.2551.

(2*S*,3*S*,4*S*)-3,4-Diacetoxy-2-[(tri-*O*-benzoyl-*α*-L-arabino-furanosyloxy)methyl]pyrrolidine (S1)

Acetic anhydride (76 µl, 0.80 mmol) was added to the diol **7** (68 mg, 0.10 mmol) in C_5H_5N (3 ml) and the solution stirred (3 h). Methanol (0.5 ml) was added and the solution kept at room temperature (10 min). The solvent was removed and flash chromatography (EtOAc/PhMe, 1:4) gave a colourless oil. This was dissolved in anhydrous CF_3CO_2H (1 ml) at 0 ˚C and the solution kept at room temperature (10 min). Concentration of the mixture and a standard workup (EtOAc, without the HCl wash), followed by

flash chromatography (EtOAc/petrol/Et_3N, 60:29:1) gave the amine **S1** as a colourless oil (41 mg, 78%), $[\alpha]_D$ +14.2. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.92, 2.06 (2 s, 6 H, $OCOCH_3$), 1.96 (bs, 1 H, NH), 3.06 (bd, 1 H, *J* = 12.7 Hz, H-5), 3.22–3.27 (m, 2 H, H-2,5), 3.77 (dd, 1 H, *J* = 3.5, 10.2 Hz, CH_2O), 4.00 (dd, 1 H, *J* = 5.5, 10.2 Hz, CH_2O), 4.65–4.72 (m, 2 H, H-4′,5′), 4.83 (dd, 1 H, *J* = 3.0, 11.5 Hz, H-5′), 5.11–5.14 (m, 2 H, H-3,4), 5.31 (s, 1 H, H-1′), 5.53 (d, 1 H, *J* = 1.4 Hz, H-2′), 5.60 (dd, 1 H, *J* = 1.4, 5.0 Hz, H-3′), 7.28–8.10 (m, 15 H, Ph); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ = 21.02, 21.03 (2 C, CH_3), 51.9 (C-5), 63.8 (C-5′), 64.0 (C-2), 66.9 (CH_2O), 77.9 (C-3′), 79.3, 79.7 (C-3,4), 81.1 (C-4′), 82.3 (C-2′), 106.0 (C-1′), 128.5–133.7 (Ph), 165.7, 165.9, 166.4, 170.2, 170.4 (5C, C=O). HRMS (FAB): m/z = 662.2243; $[M + H]^+$ requires 662.2238. Anal. Calcd. for $C_{35}H_{35}NO_{12}$: C, 63.5; H, 5.3; N, 2.1. Found: C, 63.5; H, 5.2; N, 1.8.

(2*S*,3*S*,4*S*)-3,4-Dihydroxy-1-(*tert*-butoxycarbonyl)-2-([tri-*O*-benzoyl-*α*-L-arabino-furanosyl-(1→5)-di-*O*-benzoyl-*α*-L-arabinofuranosyloxy]methyl)pyrrolidine (8)

The trichloroacetimidate **6** (0.94 g, 0.99 mmol) and the triol **4** (0.21 g, 0.90 mmol) were subjected to the general glycosylation procedure [flash chromatography (EtOAc/PhMe, 7:13)] to give the pseudo-trisaccharide **8** as a colourless oil (0.60 g, 65%), $[\alpha]_D$ +26.9. The ^{1}H and ^{13}C NMR spectra were uninterpretably broad. HRMS (FAB): m/z = 1018.3471; $[M + H]^+$ requires 1018.3497.

(2*S*,3*S*,4*S*)-3,4-Diacetoxy-2-([tri-*O*-benzoyl-*α*-L-arabino-furanosyl-(1→5)-di-*O*-benzoyl-*α*-L-arabinofuranosyloxy]-methyl)pyrrolidine (S2)

Acetic anhydride (57 µl, 0.60 mmol) was added to the diol **8** (61 mg, 0.060 mmol) in C_5H_5N (2 ml) and the solution stirred (1 h). Methanol (0.5 ml) was added and the solution kept at room temperature (10 min). The solvent was removed and flash chromatography (EtOAc/PhMe, 1:9) gave a colourless oil. This was dissolved in anhydrous CF_3CO_2H (0.5 ml) at 0 ˚C and the solution kept at room temperature (10 min). Concentration of the mixture and a standard workup (EtOAc, without the HCl wash), followed by flash chromatography (EtOAc/petrol/Et_3N, 50:49:1) gave the amine **S2** as a colourless oil (42 mg, 84%), $[\alpha]_D$ +6.0. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 1.90, 2.05 (2 s, 6 H, $OCOCH_3$), 2.07 (bs, 1 H, NH), 3.05 (bd, 1 H, *J* = 12.7 Hz, H-5), 3.20–3.25 (m, 2 H, H-2,5), 3.72 (dd, 1 H, *J* = 3.5, 10.2 Hz, CH_2O), 3.95–3.99 (m, 2 H, H-5′,CH_2O), 4.21 (dd, 1 H, *J* = 4.3, 11.3 Hz, H-5′), 4.58 (ddd, 1 H, *J* = 3.3, 4.3, 5.1 Hz, H-4′), 4.65 (dd, 1 H, *J* = 4.7, 11.9 Hz, H-5″), 4.73 (ddd, 1 H, *J* = 3.4, 4.7, 4.9 Hz, H-4″), 4.83 (dd, 1 H, *J* = 3.4, 11.9 Hz, H-5″), 5.10–5.14 (m, 2 H, H-3,4), 5.26, 5.46 (2 s, 2 H, H-1′,1″), 5.53

(d, 1 H, *J* = 1.8 Hz, H-2′), 5.58 (dd, 1 H, *J* = 1.2, 4.9 Hz, H-3″), 5.63 (d, 1 H, *J* = 1.2 Hz, H-2″), 5.68 (dd, 1 H, *J* = 1.8, 5.1 Hz, H-3′), 7.23–8.05 (m, 25 H, Ph); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ = 21.0 (CH_3), 51.9 (C-5), 63.8 (C-5″), 64.0 (C-2), 66.1 (C-5′), 66.7 (CH_2O), 77.3 (C-3′), 77.9 (C-3″), 79.2 (C-3), 79.7 (C-4), 81.4 (C-4″), 81.9 (C-4′), 82.0, 82.1 (C-2′,2″), 105.9, 106.1 (C-1′,1″), 128.4–133.7 (Ph), 165.4, 165.6, 165.8, 166.4, 170.2, 170.4 (7C, C=O). HRMS (FAB): m/z = 1002.3129; $[M + H]^+$ requires 1002.3184. Anal. Calcd. for $C_{54}H_{51}NO_{18}$: C, 64.7; H, 5.1; N, 1.4. Found: C, 64.4; H, 5.3; N, 1.4.

(2*S*,3*S*,4*S*)-2-[(*α*-L-Arabinofuranosyloxy)methyl]-3,4-dihydroxypyrrolidinium Trifluoroacetate (1.CF_3CO_2H)

Sodium (10 mg) was added to the carbamate **7** (0.34 g) in EtOH (10 ml) and the resulting solution stirred (3 h). Concentration of the mixture and flash chromatography (MeOH/EtOAc, 1:19) gave a pale yellow oil that was passed through Sephadex LH-20 (H_2O/MeOH, 1:4) to return a colourless oil. The thoroughly dried product was dissolved in anhydrous CF_3CO_2H (1.5 ml) at 0 ˚C and the solution kept at room temperature (10 min). The solvent was completely removed (r.t.) and the residue lyophilized from H_2O (2 ml) to give the pyrrolidinium trifluoroacetate **1.CF_3CO_2H** as a colourless glass (133 mg, 73%), $[\alpha]_D$ –38.0. ^{1}H NMR (600 MHz, D_2O) δ = 3.36 (dd, 1 H, *J* = 3.0, 12.5 Hz, H-5), 3.60 (dd, 1 H, *J* = 5.0, 12.5 Hz, H-5), 3.70 (dd, 1 H, *J* = 6.1, 12.2 Hz, H-5′), 3.76 (ddd, 1 H, *J* = 4.2, 4.2, 8.0 Hz, H-2), 3.82 (dd, 1 H, *J* = 3.3, 12.2 Hz, H-5′), 3.92 (dd, 1 H, *J* = 4.2, 11.4 Hz, CH_2O), 3.96 (dd, 1 H, *J* = 3.0, 5.7 Hz, H-3′), 4.01 (dd, 1 H, *J* = 8.0, 11.4 Hz, CH_2O), 4.08–4.16 (m, 3 H, *J* = Hz, H-3,2′,4′), 4.34 (ddd, 1 H, *J* = 3.0, 3.0, 5.0 Hz, H-4), 5.09 (d, 1 H, *J* = 1.2 Hz, H-1′); ^{13}C NMR (150.9 MHz, D_2O) δ = 50.6 (C-5), 61.9 (C-5′), 65.0 (2 C, C-2,CH_2O), 74.8 (C-4), 76.4 (C-3), 77.1 (C-3′), 81.5 (C-2′), 85.0 (C-4′), 107.8 (C-1′), 117.0 (q, *J* = 292 Hz, CF_3), 163.6 (q, *J* = 35.5 Hz, C=O). HRMS (FAB): m/z = 266.1251; $[M - CO_2CF_3]^+$ requires 266.1240. Anal. Calcd. for $C_{12}H_{20}F_3NO_9$: C, 38.0; H, 5.3; N, 3.7. Found: C, 37.8; H, 5.5a; N, 3.6.

(2*S*,3*S*,4*S*)-2-([*α*-L-Arabinofuranosyl-(1→5)-*α*-L-arabinofuranosyloxy]methyl)-3,4-dihydroxypyrrolidinium Trifluoroacetate (2.CF_3CO_2H)

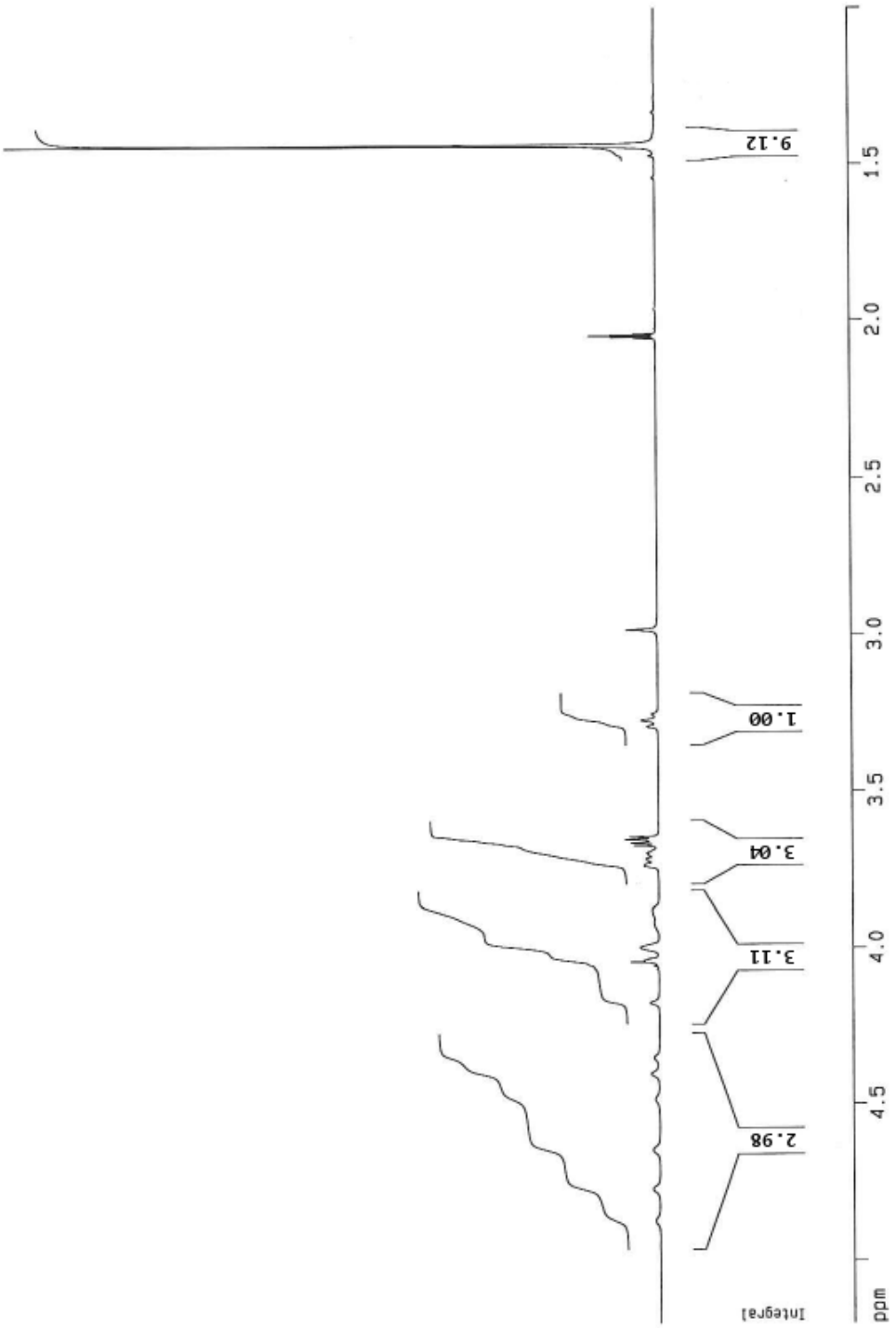
Sodium (10 mg) was added to the carbamate **8** (0.51 g) in EtOH (10 ml) and the resulting solution stirred (3 h). Concentration of the mixture and flash chromatography (MeOH/EtOAc, 1:9) gave a pale yellow oil that was passed through Sephadex LH-20 (H_2O/MeOH, 1:3) to return a colourless oil. The

thoroughly dried product was dissolved in anhydrous CF_3CO_2H (1.5 ml) at 0˚C and the solution kept at room temperature (10 min). The solvent was completely removed (r.t.) and the residue lyophilized from H_2O (2 ml) to give the pyrrolidinium trifluoroacetate **2.CF_3CO_2H** as a colourless glass (0.19 g, 75%), $[\alpha]_D$ –38.0. ^{1}H NMR (600 MHz, D_2O) δ = 3.39 (dd, 1 H, *J* = 3.0, 12.5 Hz, H-5), 3.63 (dd, 1 H, *J* = 5.0, 12.5 Hz, H-5), 3.74 (dd, 1 H, *J* = 5.9, 12.3 Hz, H-5″), 3.79 (ddd, 1 H, *J* = 4.0, 4.2, 8.0 Hz, H-2), 3.82 (dd, 1 H, *J* = 3.3, 11.4 Hz, H-5′), 3.85 (dd, 1 H, *J* = 3.3, 12.3 Hz, H-5″), 3.91 (dd, 1 H, *J* = 5.9, 11.4 Hz, H-5′), 3.95 (dd, 1 H, *J* = 4.2, 11.2 Hz, CH_2O), 3.98 (dd, 1 H, *J* = 3.3, 6.0 Hz, H-3″), 4.02–4.07 (m, 2 H, H3′,CH_2O), 4.11 (ddd, 1 H, *J* = 3.3, 5.9, 6.0 Hz, H-4″), 4.14 (dd, 1 H, *J* = 1.6, 3.3 Hz, H-2″), 4.17–4.19 (m, 2 H, H-3,2′), 4.25 (ddd, 1 H, *J* = 3.3, 5.9, 5.9 Hz, H-4′), 4.37 (ddd, 1 H, *J* = 3.0, 3.0, 5.0 Hz, H-4), 5.11 (d, 1 H, *J* = 1.6 Hz, H-1″), 5.14 (d, 1 H, *J* = 1.3 Hz, H-1′); ^{13}C NMR (150.9 MHz, D_2O) δ = 50.6 (C-5), 61.8 (C-5″), 65.0 (C-2), 65.1 (CH_2O), 67.5 (C-5′), 74.8 (C-4), 76.5 (C-3), 77.1 (C-3″), 77.3 (C-3′), 81.4 (C-2′), 81.5 (C-2″), 83.4 (C-4′), 84.6 (C-4″), 107.9 (C-1′), 108.1 (C-1″), 117.0 (q, *J* = 292 Hz, CF_3), 163.6 (q, *J* = 35.5 Hz, C=O). HRMS (FAB): m/z = 398.3841; $[M - CO_2CF_3]^+$ requires 398.3829. Anal. Calcd. for $C_{17}H_{28}F_3NO_{13}$: C, 39.9; H, 5.5; N, 2.7. Found: C, 40.0; H, 5.5; N, 2.7.

NMR Spectra

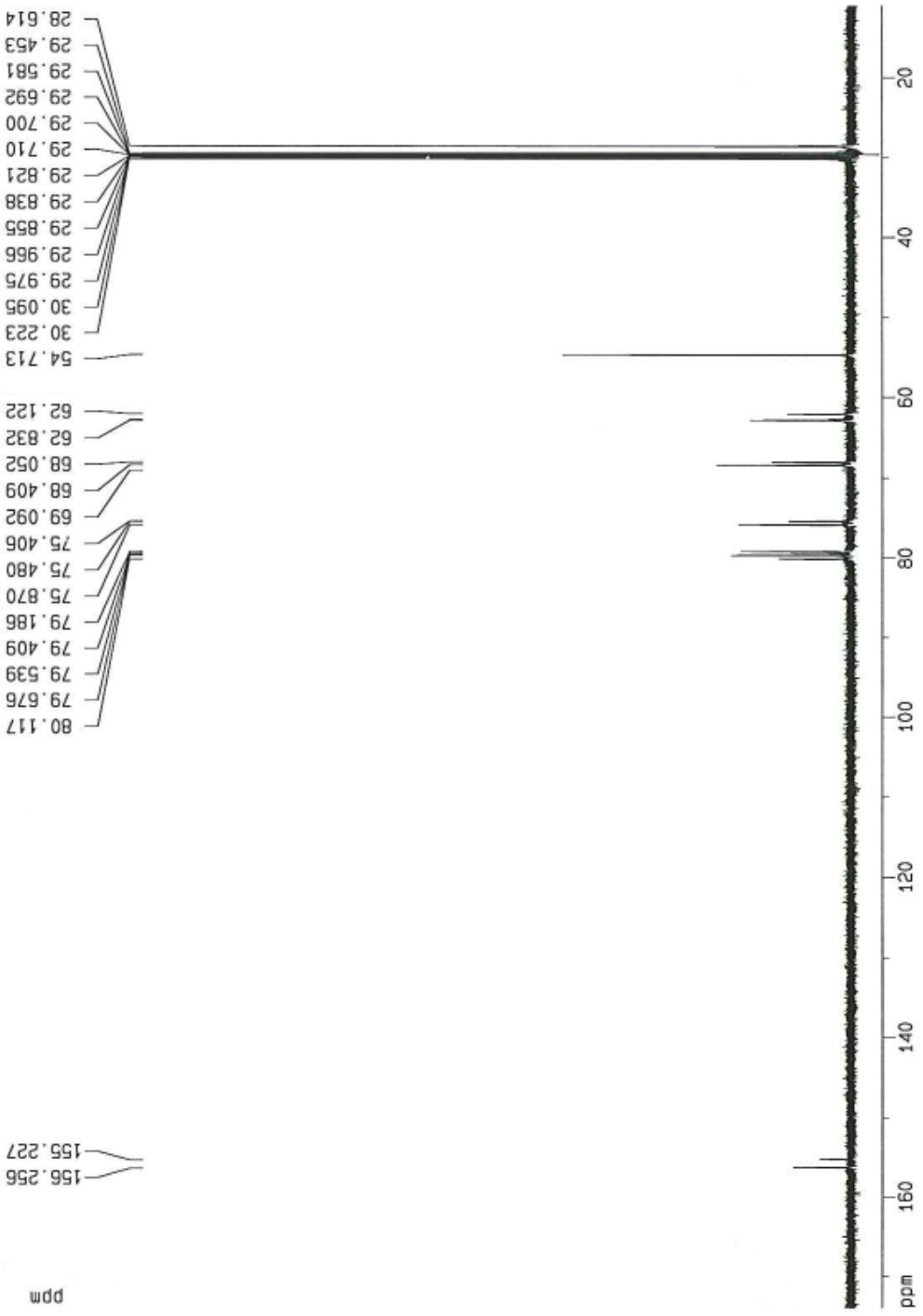
(2*S*,3*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-3,4-dihydroxy-2-(hydroxymethyl)pyrrolidine (4)

^{1}H NMR [600 MHz, $(CD_3)_2CO$]

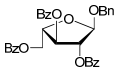


(2*S*,3*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-3,4-dihydroxy-2-(hydroxymethyl)pyrrolidine (4)

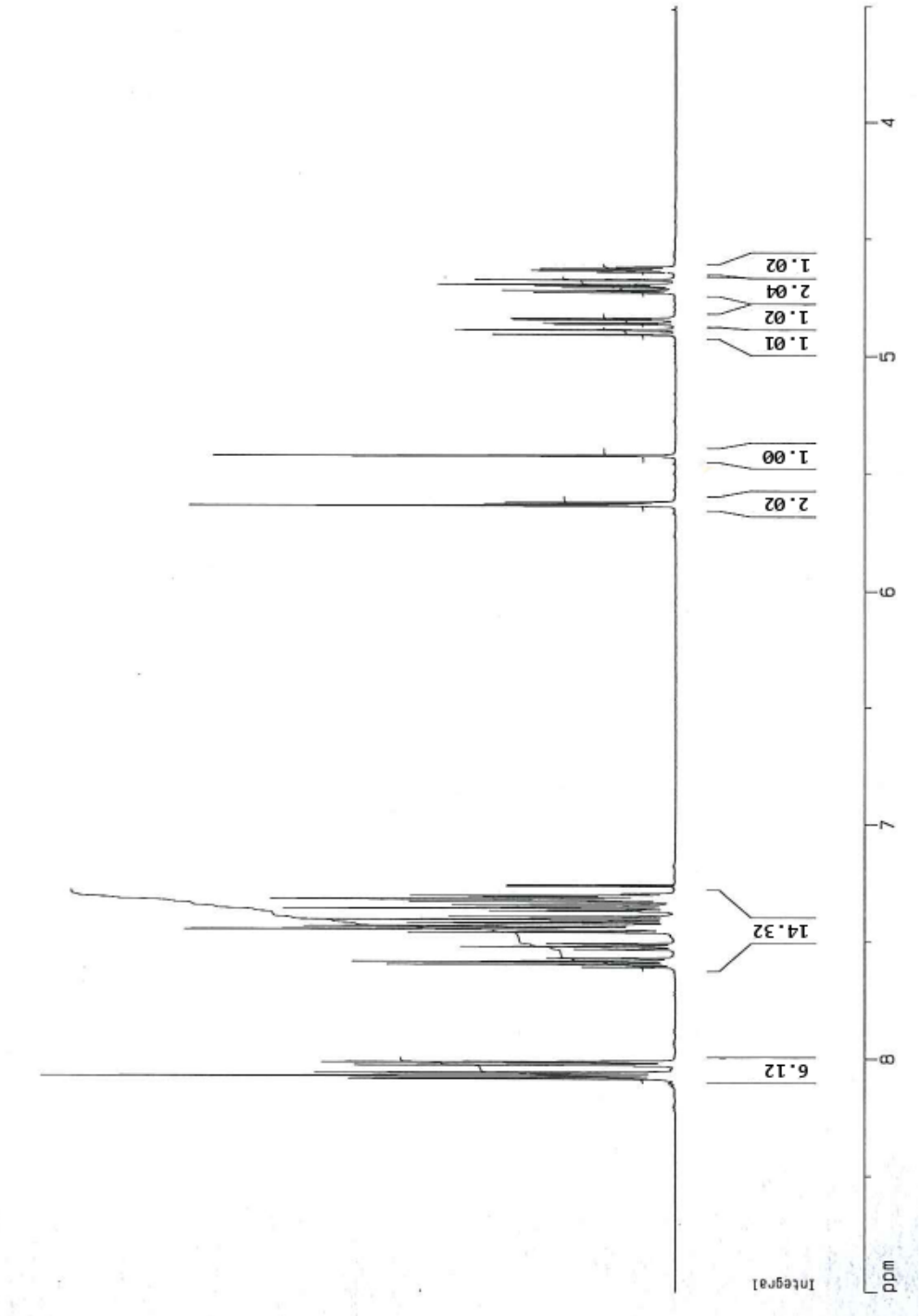
^{13}C NMR [150.9 MHz, $(CD_3)_2CO$]



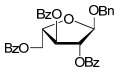
Benzyl 2,3,5-Tri-*O*-benzoyl-*α*-L-arabinoside



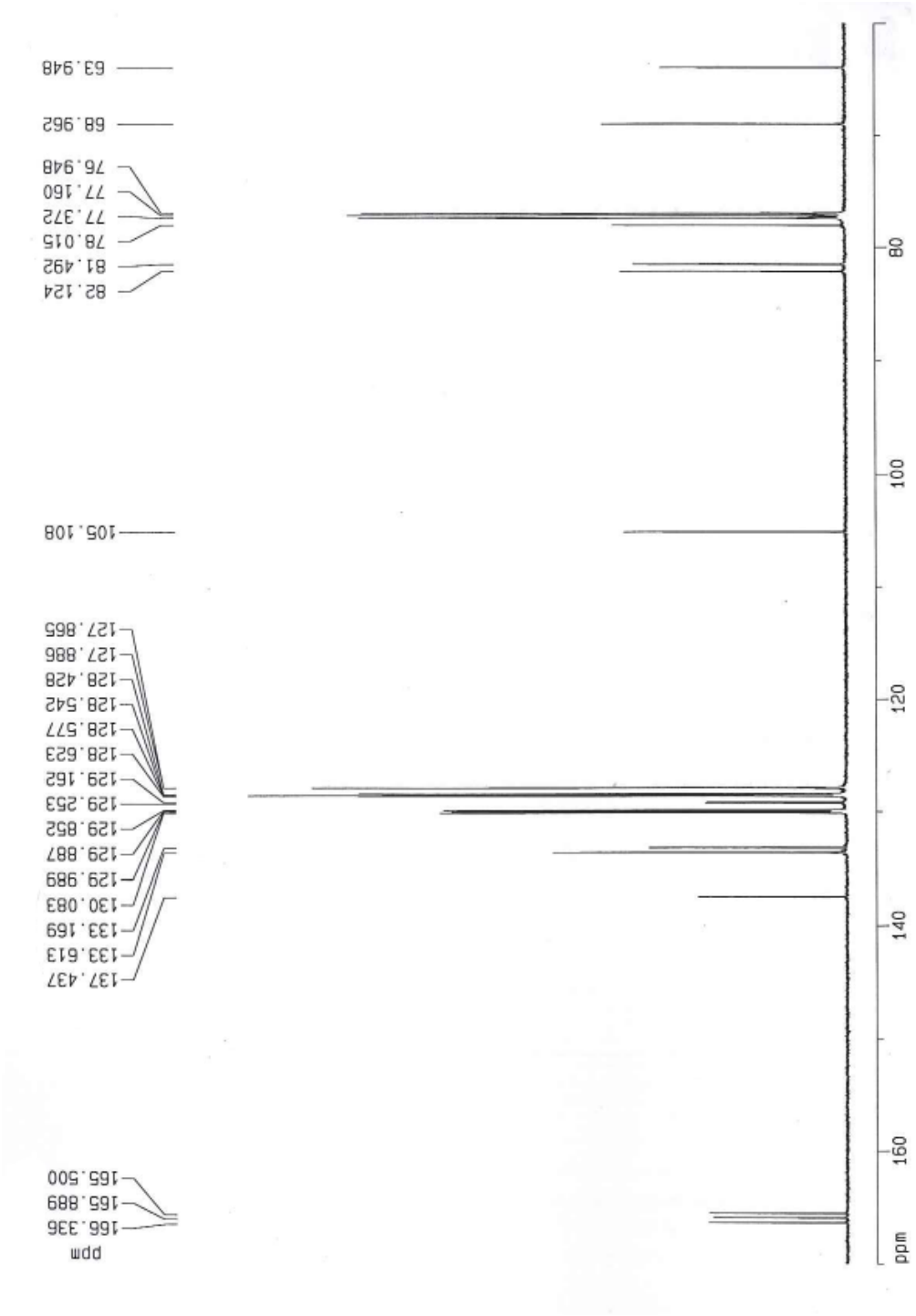
^{1}H NMR (600 MHz, $CDCl_3$)



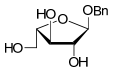
Benzyl 2,3,5-Tri-*O*-benzoyl-*α*-L-arabinoside



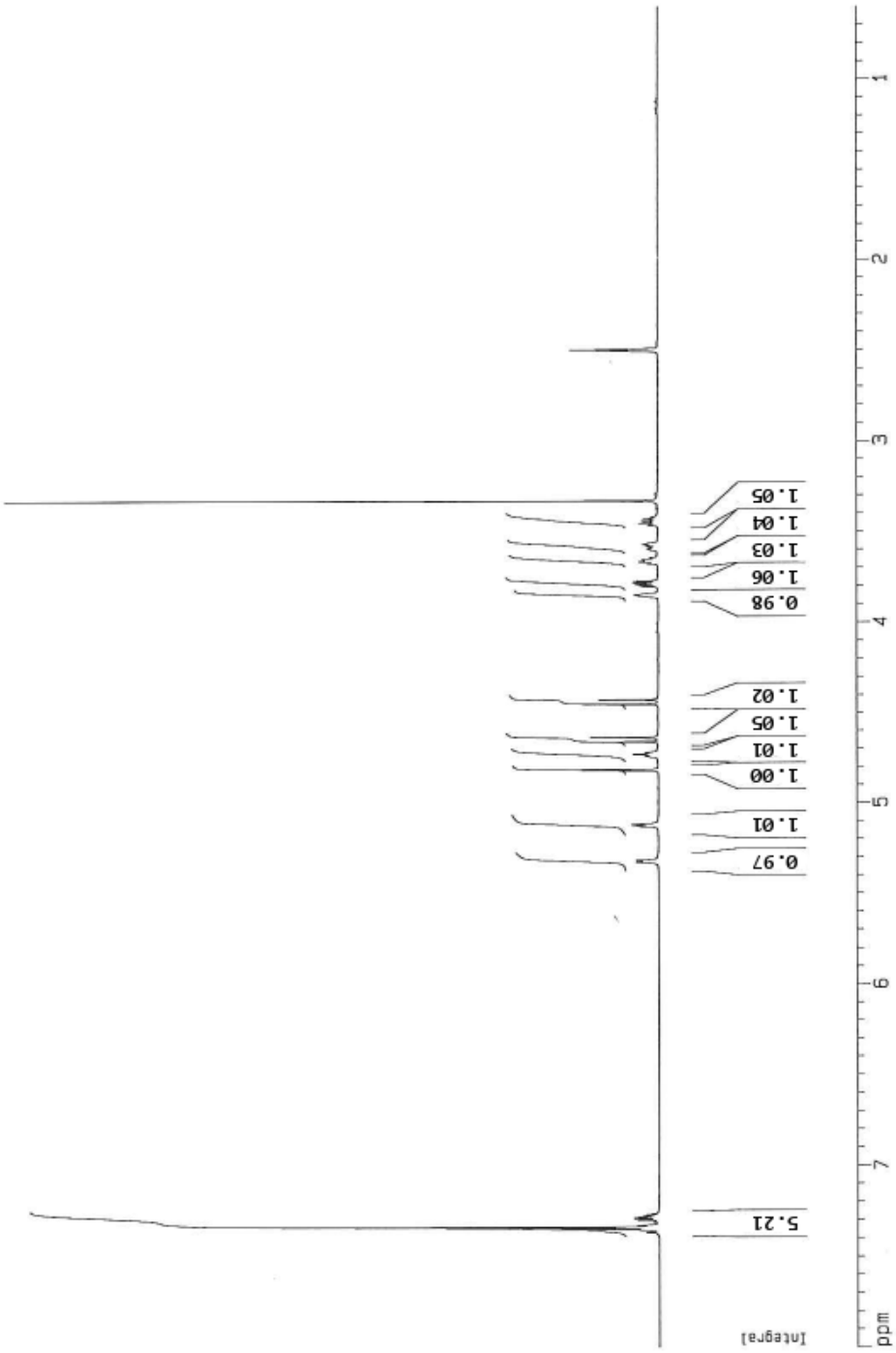
^{13}C NMR (150.9 MHz, $CDCl_3$)



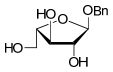
Benzyl *α*-L-Arabinofuranoside



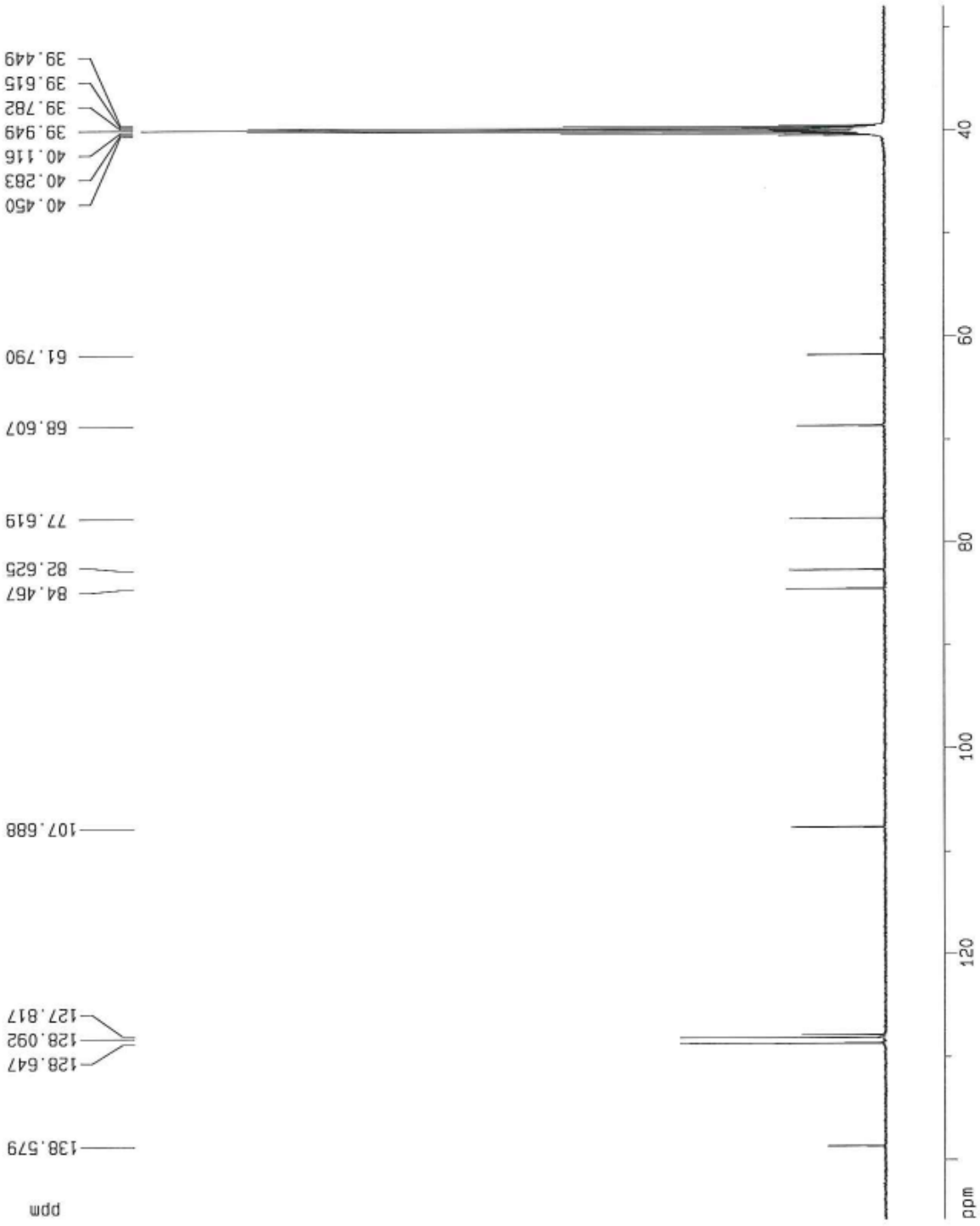
^{1}H NMR [500 MHz, $(CD_3)_2SO$]



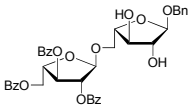
Benzyl *α*-L-Arabinofuranoside



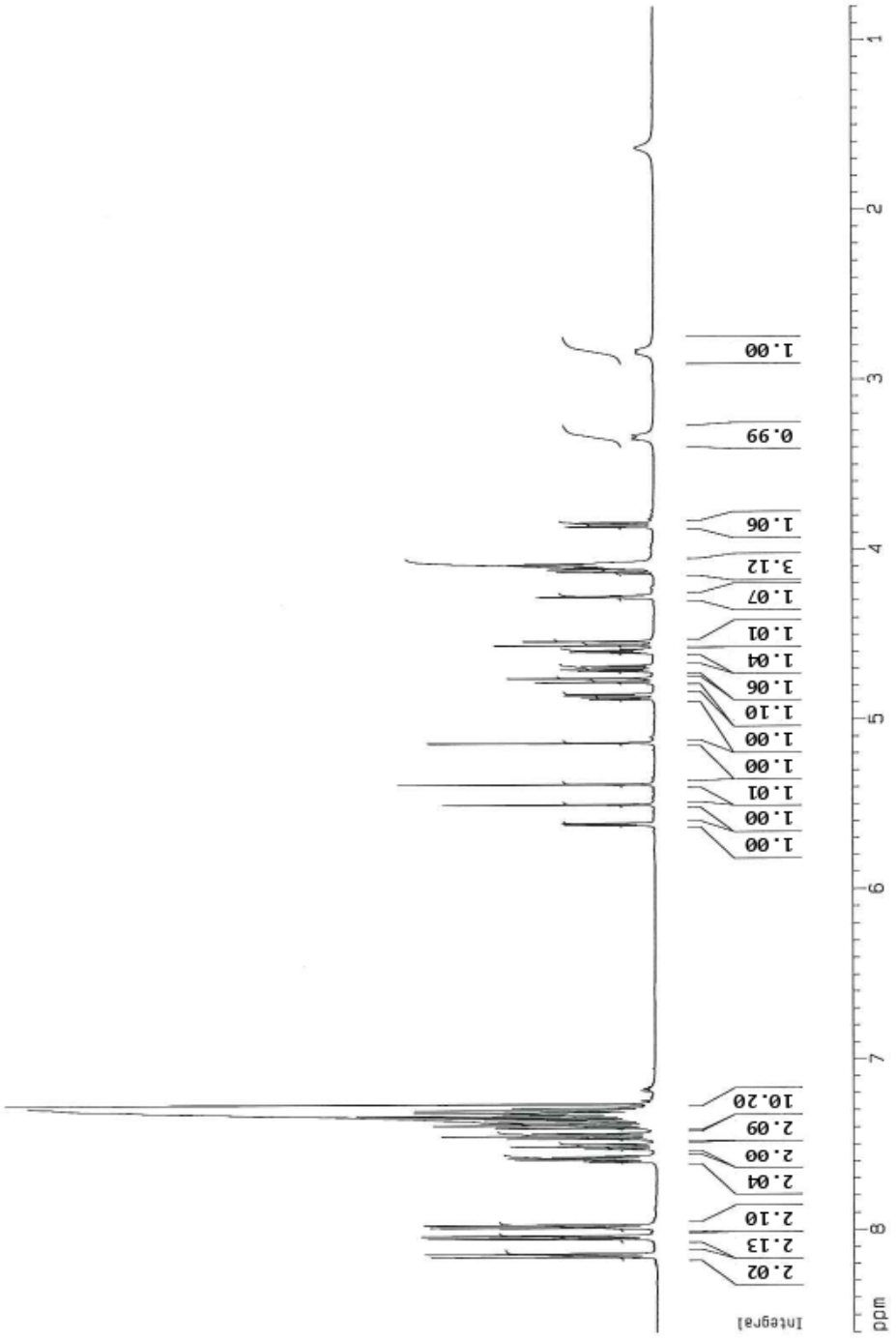
^{13}C NMR [125.8 MHz, $(CD_3)_2SO$]



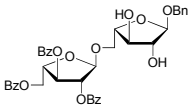
Benzyl Tri-*O*-benzoyl-*α*-L-arabinofuranosyl-(1→5)-*α*-L-arabinofuranoside



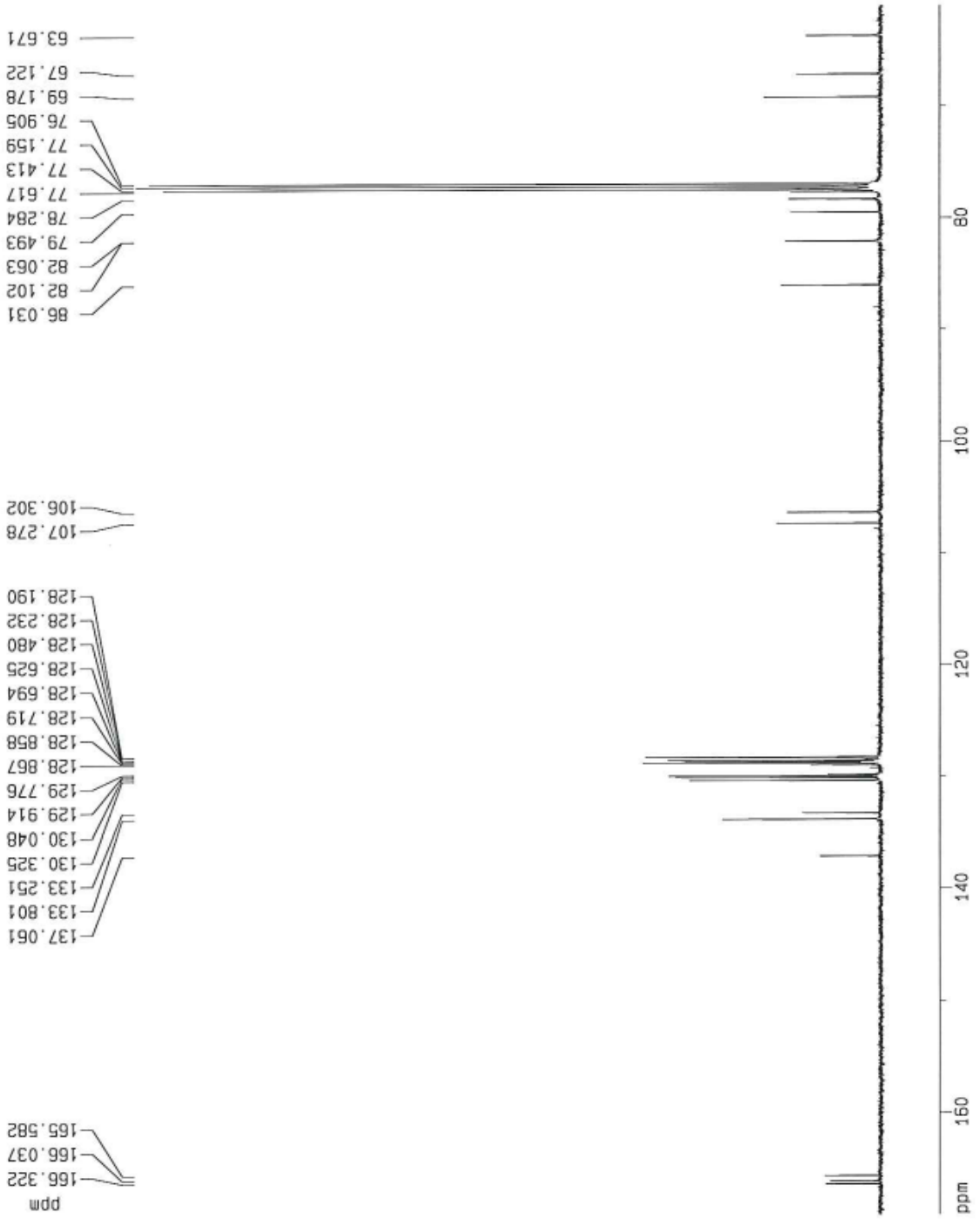
^{1}H NMR (500 MHz, $CDCl_3$)



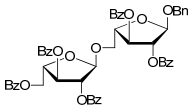
Benzyl Tri-*O*-benzoyl-*α*-L-arabinofuranosyl-(1→5)-*α*-L-arabinofuranoside



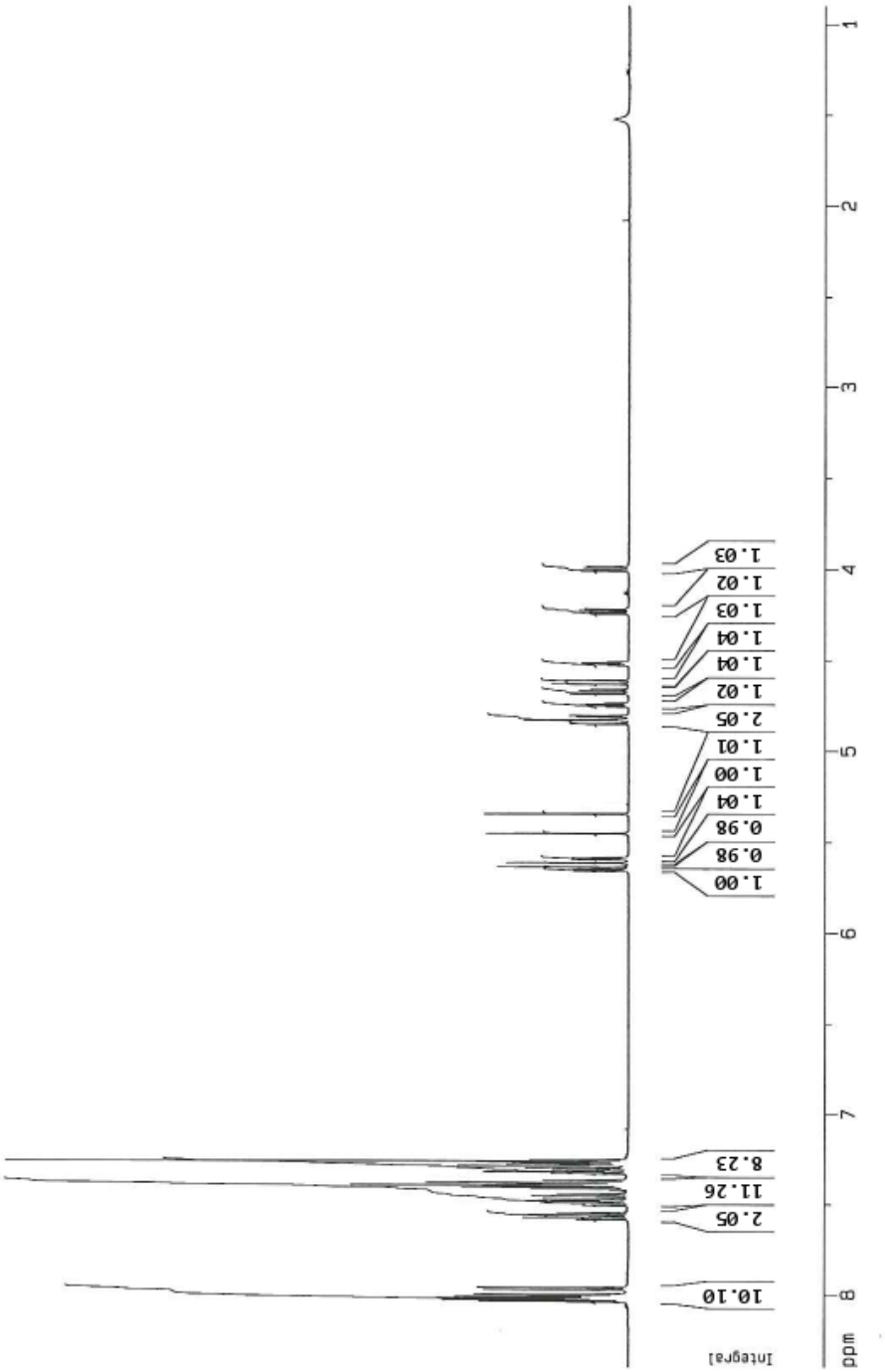
^{13}C NMR (125.8 MHz, $CDCl_3$)



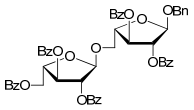
Benzyl Tri-*O*-benzoyl-*α*-L-arabinofuranosyl-(1→5)-di-*O*-benzoyl-*α*-L-arabinofuranoside



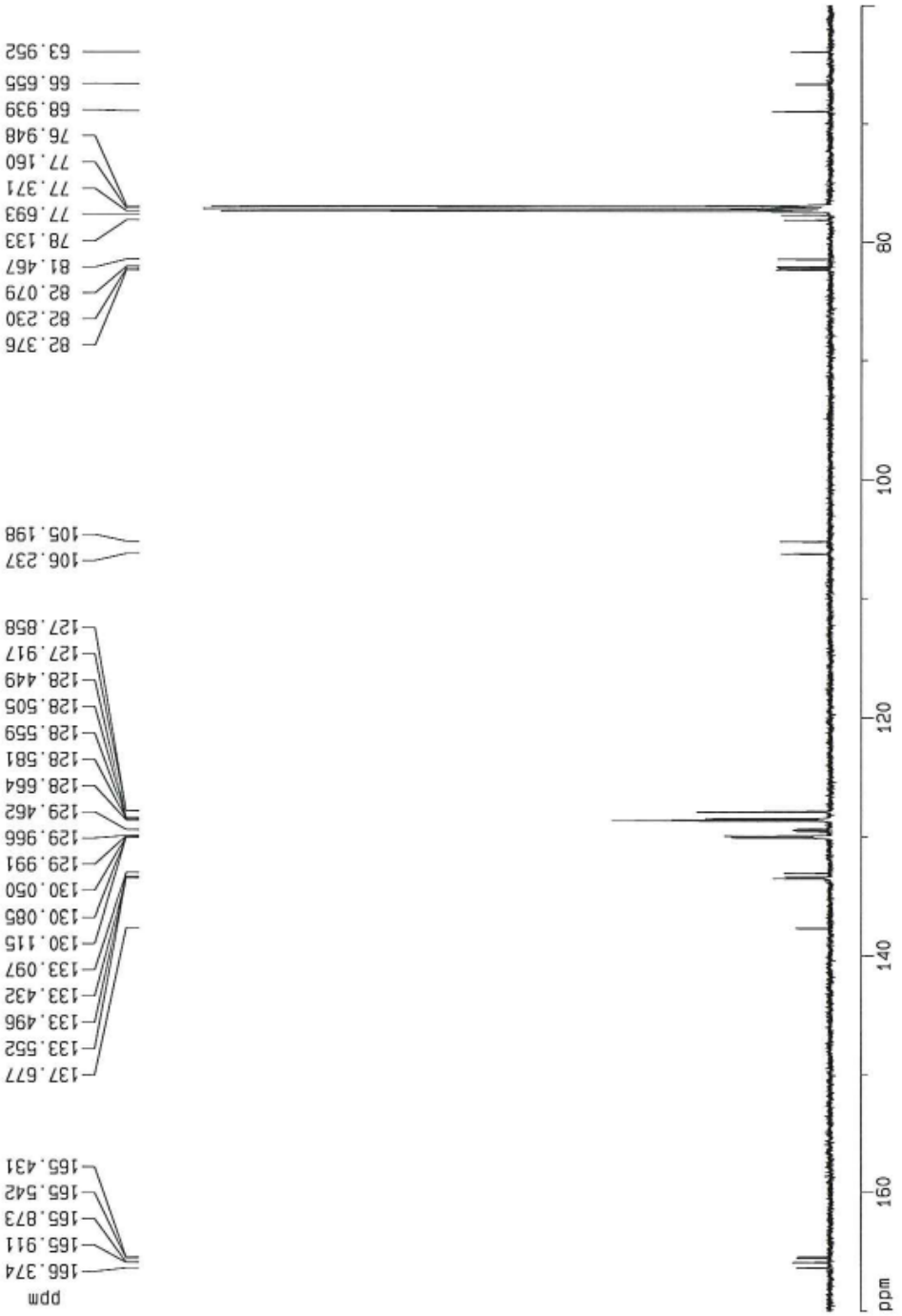
^{1}H NMR (600 MHz, $CDCl_3$)



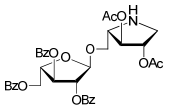
Benzyl Tri-*O*-benzoyl-*α*-L-arabinofuranosyl-(1→5)-di-*O*-benzoyl-*α*-L-arabinofuranoside



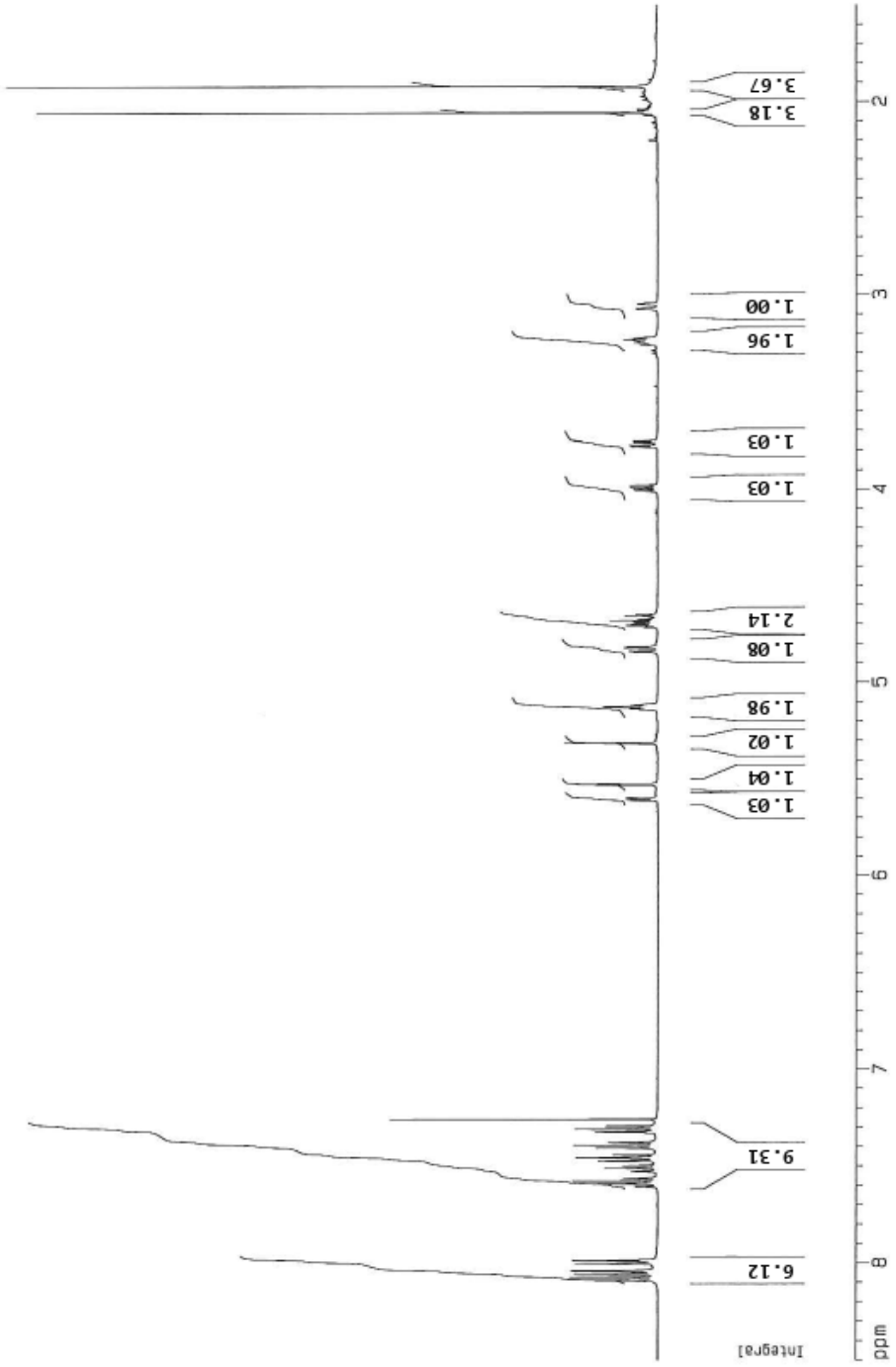
^{13}C NMR (150.9 MHz, $CDCl_3$)



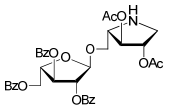
(2*S*,3*S*,4*S*)-3,4-Diacetoxy-2-[(tri-*O*-benzoyl-*α*-L-arabinofuranosyloxy)methyl]pyrrolidine (S1)



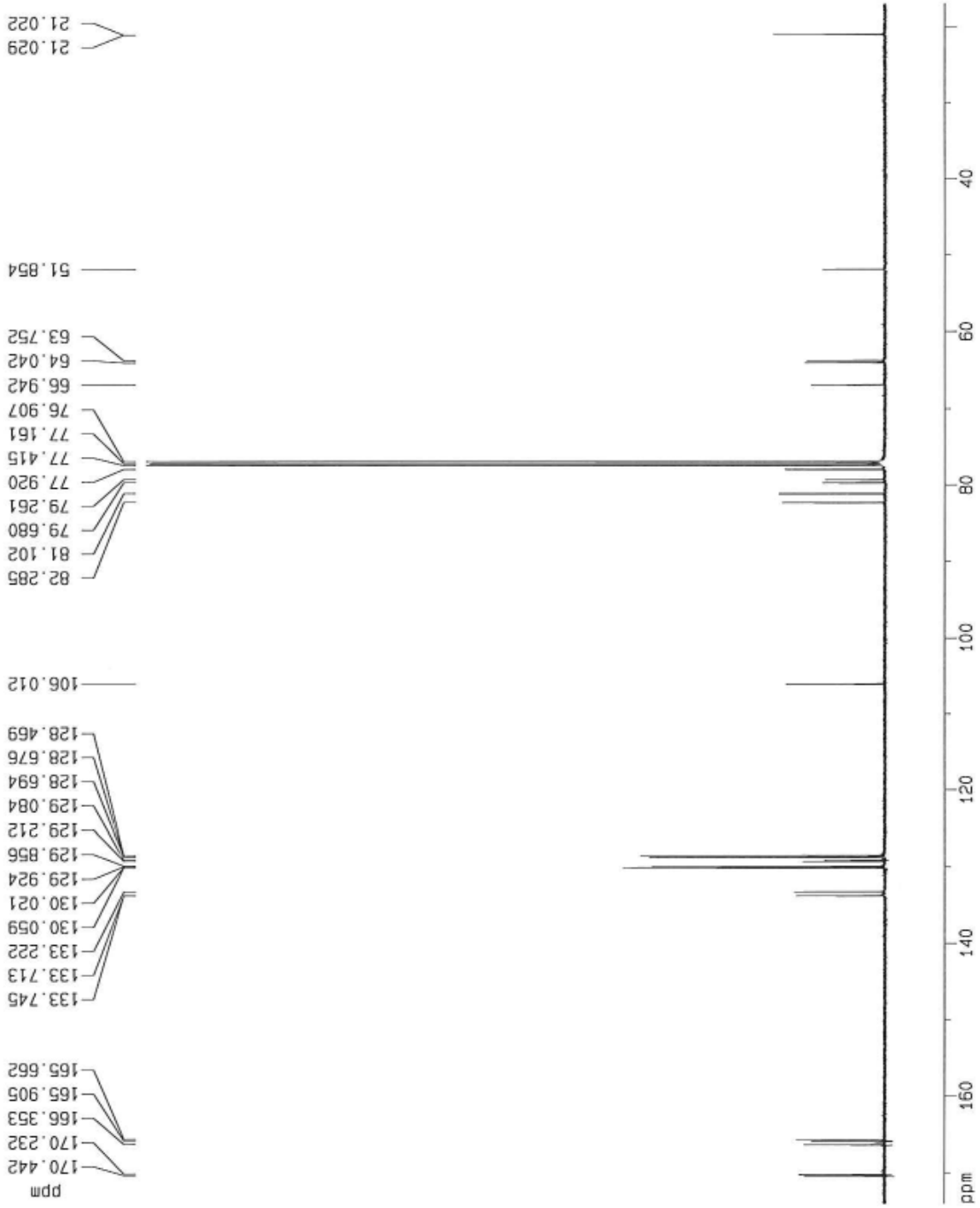
^{1}H NMR (500 MHz, $CDCl_3$)



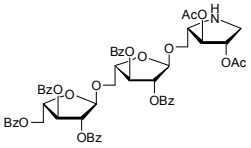
(2*S*,3*S*,4*S*)-3,4-Diacetoxy-2-[(tri-*O*-benzoyl-*α*-L-arabinofuranosyloxy)methyl]pyrrolidine (S1)



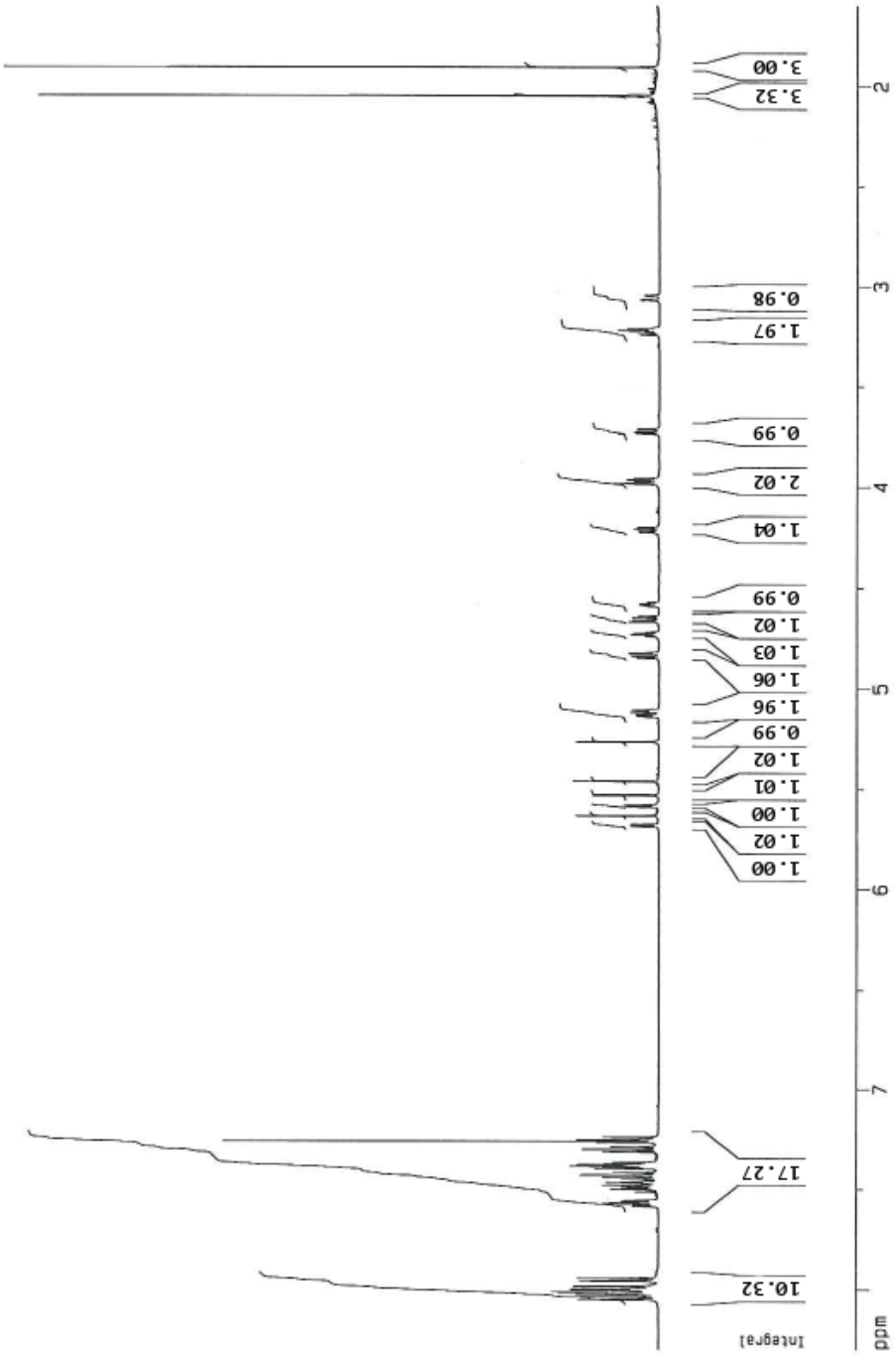
^{13}C NMR (125.8 MHz, $CDCl_3$)



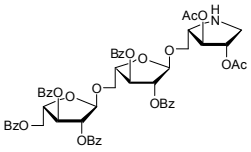
(2*S*,3*S*,4*S*)-3,4-Diacetoxy-2-([tri-*O*-benzoyl-*α*-L-arabinofuranosyl-(1→5)-di-*O*-benzoyl-*α*-L-arabinofuranosyloxy]-methyl)pyrrolidine (S2)



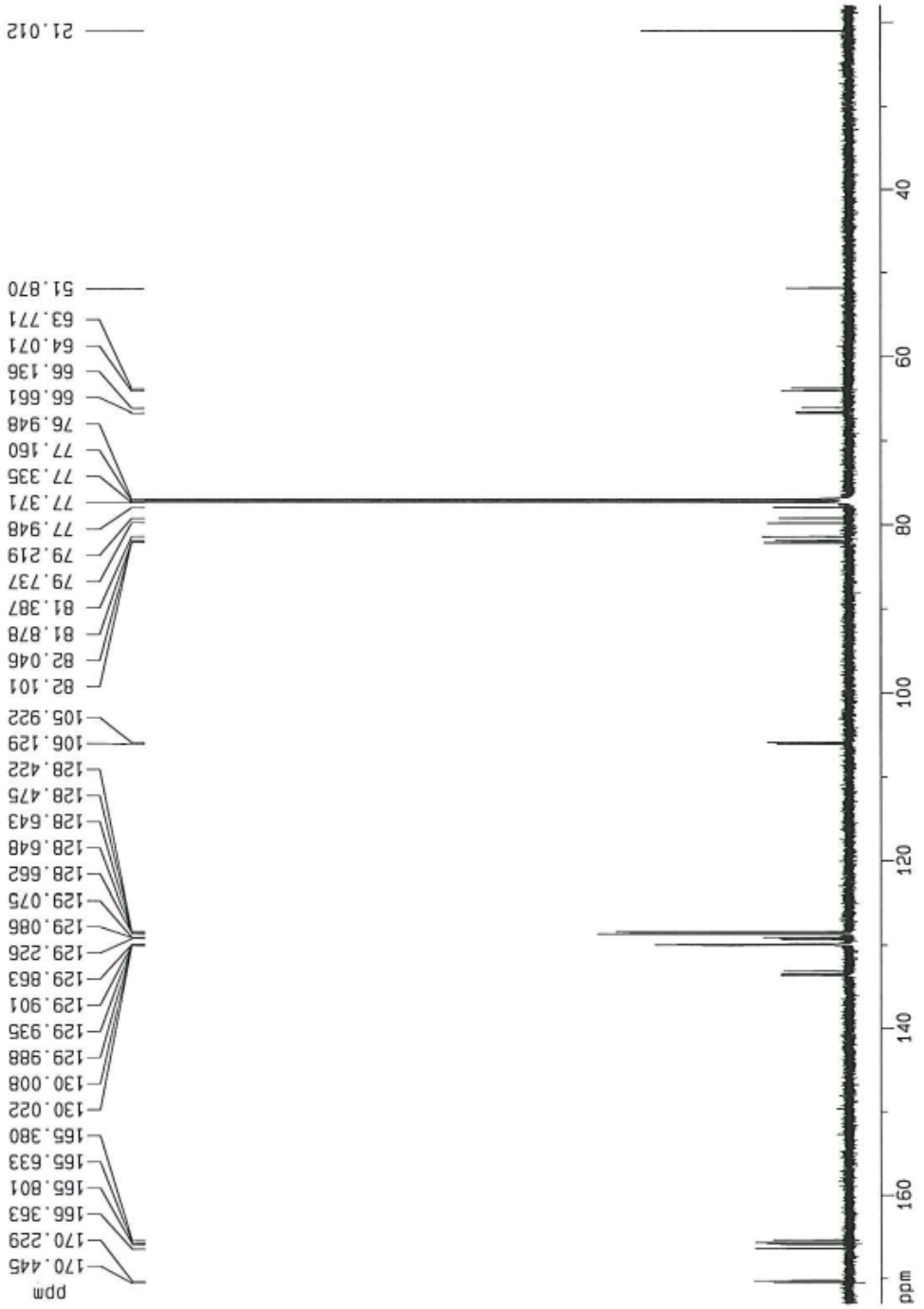
^{1}H NMR (600 MHz, $CDCl_3$)



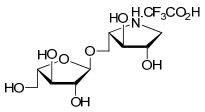
(2*S*,3*S*,4*S*)-3,4-Diacetoxy-2-([tri-*O*-benzoyl-*α*-L-arabinofuranosyl-(1→5)-di-*O*-benzoyl-*α*-L-arabinofuranosyloxy]-methyl)pyrrolidine (S2)



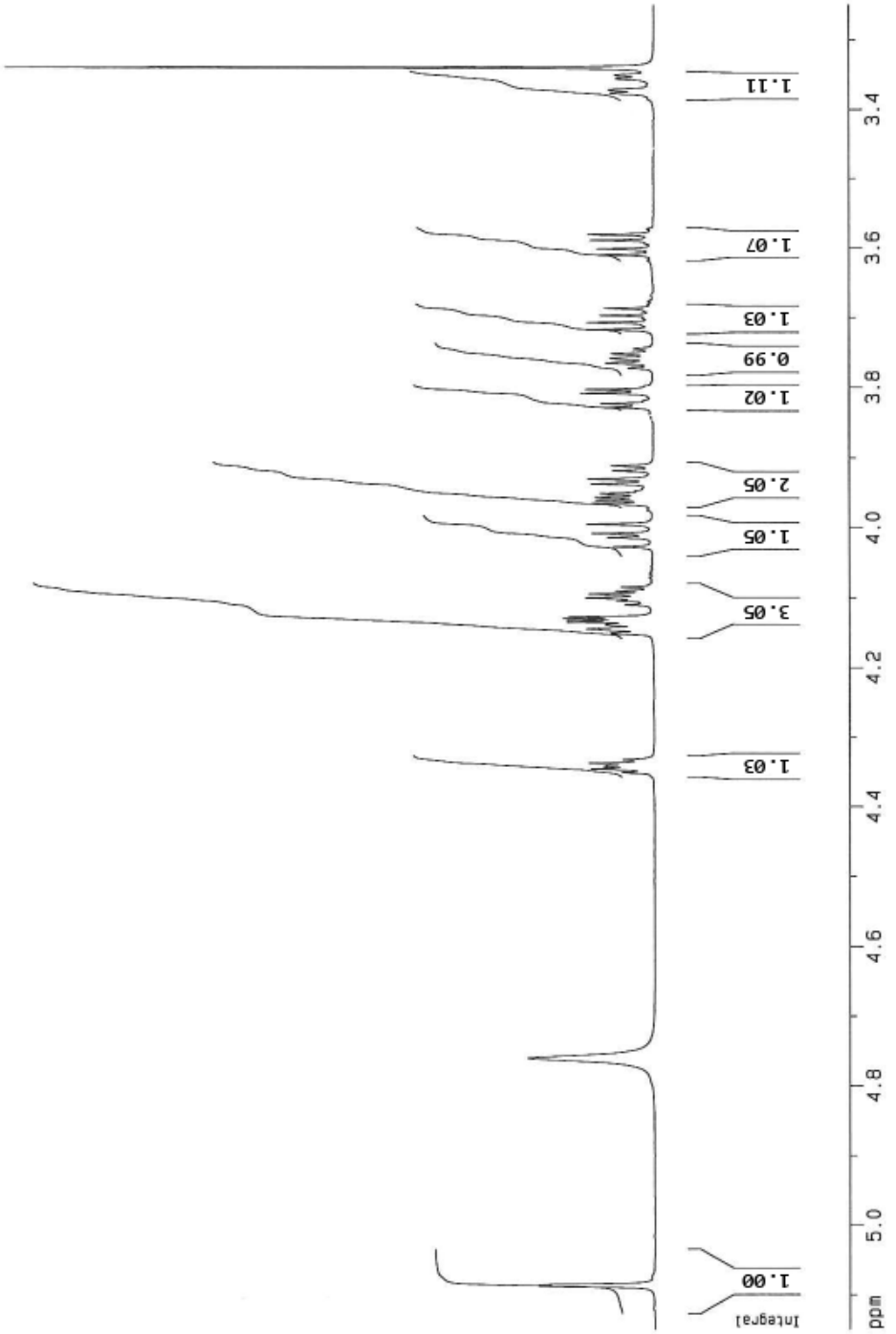
^{13}C NMR (125.8 MHz, $CDCl_3$)



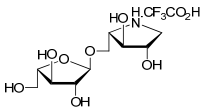
(2*S*,3*S*,4*S*)-2-[(*α*-L-Arabinofuranosyloxy)methyl]-3,4-dihydroxypyrrolidinium Trifluoroacetate (1.CF_3CO_2H)



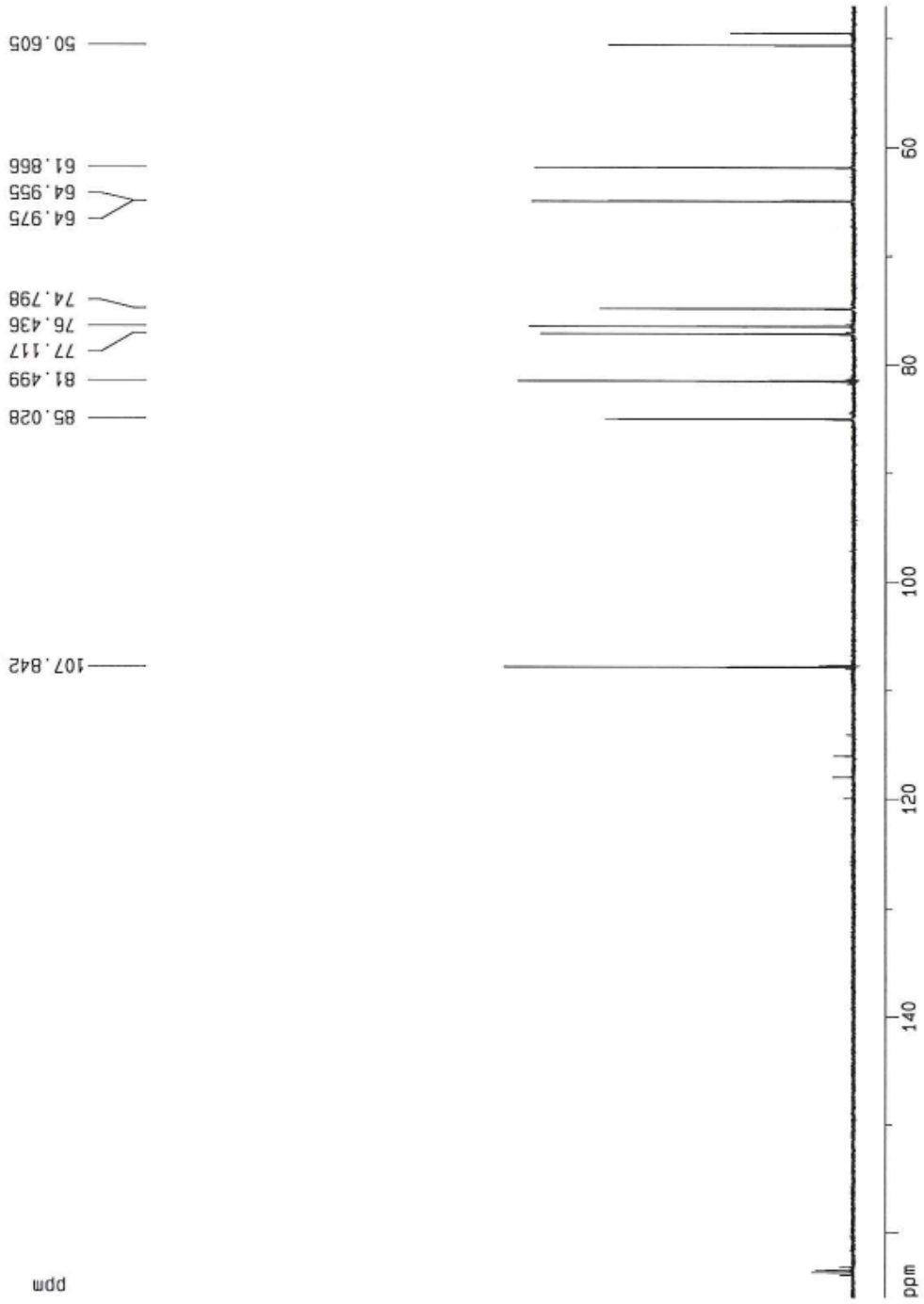
^{1}H NMR (600 MHz, D_2O)



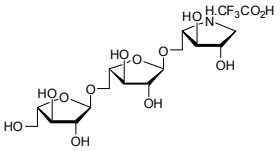
(2*S*,3*S*,4*S*)-2-[(*α*-L-Arabinofuranosyloxy)methyl]-3,4-dihydroxypyrrolidinium Trifluoroacetate (1.CF_3CO_2H)



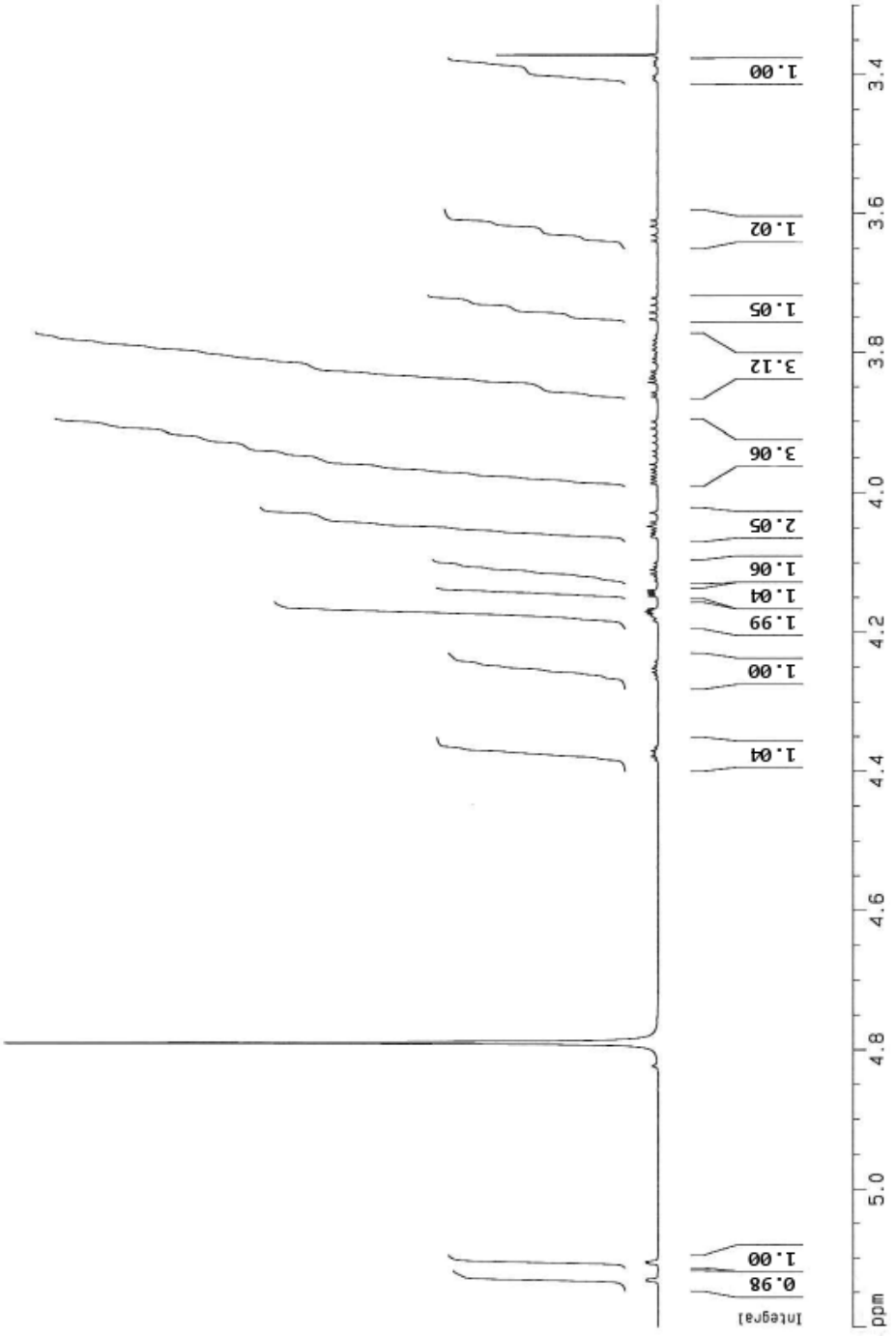
^{13}C NMR (150.9 MHz, D_2O)



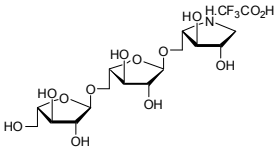
(2*S*,3*S*,4*S*)-2-([*α*-L-Arabinofuranosyl-(1→5)-*α*-L-arabinofuranosyloxy]methyl)-3,4-dihydroxypyrrolidinium Trifluoroacetate (2.CF_3CO_2H)



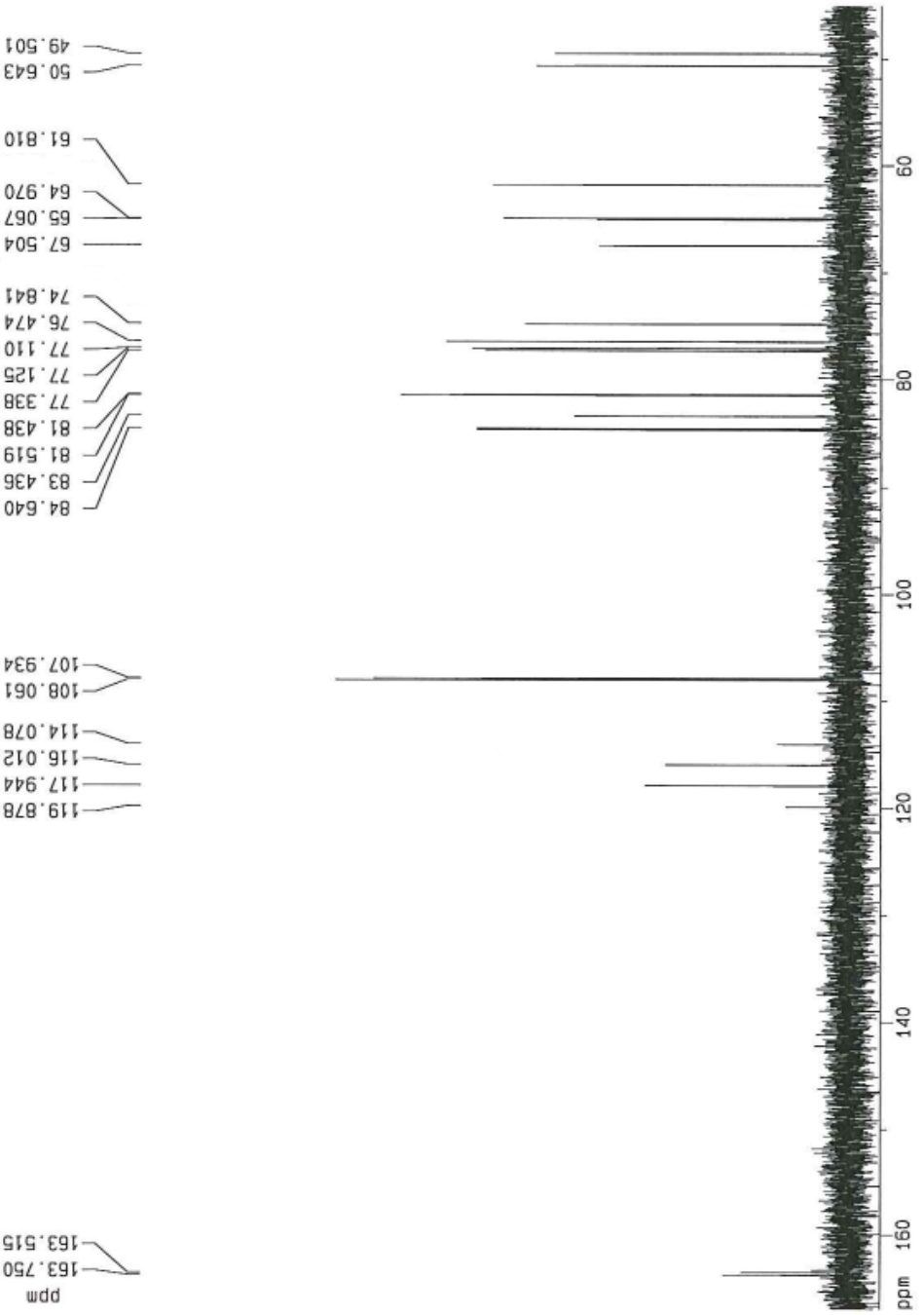
^{1}H NMR (600 MHz, D_2O)



(2*S*,3*S*,4*S*)-2-([*α*-L-Arabinofuranosyl-(1→5)-*α*-L-arabinofuranosyloxy]methyl)-3,4-dihydroxypyrrolidinium Trifluoroacetate (2.CF_3CO_2H)



^{13}C NMR (150.9 MHz, D_2O)



Structures Determination

The proteins were expressed and purified as described previously[1]. Samples of Arb93A wild-type and E242A mutant (~12 $mg.ml^{-1}$) were incubated with 1.0 and 0.1 mM inhibitor for 1 h. prior to crystallisation using 100 mM Hepes buffer at pH 7.5, 100 mM $MnSO_4$ and 38% PEG 550MME and 100 mM Hepes buffer at pH 7.5 and 40% PEG 550MME, respectively. Crystals were mounted in a rayon fiber loop in a N_2 stream at 100 K. Data were collected from a single crystal at the European Synchrotron Radiation Facility, on beam-line BM30A (wild-type complex) and ID14-4 (mutant complex) using an ADSC charge-coupled device. Data were processed using XDS[2] for the wild type complex and MOSFLM[3] for the mutant complex. All further computing was performed using the CCP4 suite[4] unless otherwise stated. Both structures were solved by molecular replacement using the program PHASER[5] and the native wild-type coordinates (PDB: 2W5N) as a search model. The structures were refined using REFMAC[6] iterated with manual rebuilding in COOT[6] . The incorporation of the ligand **1** was performed after inspection of the mFo−DFc weighted maps. Water molecules were introduced automatically using Coot and inspected manually. The coordinates were deposited in the Protein Data bank under the code 2YDT and 2YDP for the native and mutant complex structures, respectively. Details of the models quality are given in the Table S1.

(1) R. Carapito, A. Imberty, J. M. Jeltsch, S. C. Byrns, P. H. Tam, T. L. Lowary, A. Varrot and V. Phalip, *J. Biol. Chem.*, 2009, **284**, 12285.
(2) Kabsch, W. *Acta Crystallogr. D Biol. Crystallogr.* 2010, **66**, 125.
(3) Leslie, A. G. W. *Jnt CCP4/ESF-EACMB. Newsletter on Protein Crystallography,* 1992, **26**.
(4) Collaborative Computational Project Number 4, *Acta Crystallogr.* 1994, **D50**, 760.
(5) McCoy, A. J.; *et al. J. Appl. Crystallogr.* 2007, **40**, 658.
(6) Murshudov, G.N.; Vagin, A. A.; Dodson, E. J. *Acta Crystallogr. D Biol. Crystallogr.* 1997**, 53**, 240.
(7) Emsley, P.; Cowtan, K. *Acta Crystallogr. D Biol. Crystallogr.* 2004, **60**, 2126.

Table S1. Data collection and refinement statistics

Data set	**Wildtype-(1) complex**	**E242A-(1) complex**
Spacegroup	$I4_122$	P1
Unit cell	105.43 105.43 140.04 90.0 90.0 90.0	56.93 57.80 91.60 90.14 93.55 114.5
Resolution (outer shell), Å	44.69-1.60 (1.69-1.60)	33.97-1.85 (1.95-1.85)
Meas/ Unique reflections	393852/52113	168942/86184
Multiplicity	7.6 (5.0)	2.0 (2.0)
Completeness, %	99.9 (99.5)	95.2 (95.2)
R_{merge}	0.061 (0.379)	0.101 (0.372)
Mean I/σI	23.1 (4.0)	6.4 (2.4)
Wilson plot B	13.8	18.9
Refinement		
R_{cryst} / R_{free}	14.2/17.0	17.1/22.6
Rmsd bonds, Å/angles, °/chiral	0.016/1.60/0.101	0.0144/1.528/0.101
protein atoms/ Bfac, $Å^2$	2919/11.6	A:2876/17.7 B: 2859/22.6 C:2844/22.1
ligand atoms/ Bfac, $Å^2$	18/7.3	A:18/14.4 B: 18/21.0 C:18/17.4
water molecules/ Bfac, $Å^2$	524/25.3	A:401/28.0 B: 275/29.5 C:288/28.8
PDB code	2YDT	2YDP

* Values in parentheses refer to the highest resolution shell.

K_i determination

Kinetics studies were performed with debranched arabinan (Megazyme) as substrate. Arb93A was incubated at 6.57 µM in 50 mM sodium acetate buffer, pH5 at 40 °C with substrate concentrations ranging from 2.31 mM to 9.02 µM in presence or absence e of 10.4 µM of compound **1**. The reactions were stopped after 5 min by heating to 95 °C, dried using a centrifugal vacuum evaporator (SpeedVac, Thermo Scientific) and labeled with fluorophore 8-aminonaphthalene-1,3,6-trisulfonic acid for PACE (polysaccharide analysis using gel electrophoresis) as described previously[1] (Carapito et al., 2009). After electrophoresis, fluorescent hydrolysis products of arabinan (arabinobiose and arabinose) were visualized under UV-light using a Gel Doc XR device (BioRad) and intensities of bands were quantified with the QuantityOne software (adjusted volumes after background subtraction). All reactions were performed in duplicate and data represent the mean +/- standard deviation. Kinetics parameters were derived from Lineweaver-Burk plots and K_i values were calculated using the formula $K_{Mapp} = K_M (1 + [I] / K_i)$ where K_{Mapp} is the K_M measured in the presence of the inhibitor **1**.

(1) Carapito, R., Carapito, C., Jeltsch, J. M., and Phalip, V. *Biores. Technol.* 2009, **100**, 845.