Supporting Materials for

Large-Scale Synthesis of a Substituted D-Phenylalanine Using Asymmetric Hydrogenation.

by

Martin E. Fox,[a*] Mark Jackson,[a] Graham Meek[a] and Matthew Willets[b]

[a] *Chirotech Technology Centre, Dr Reddy's Laboratories EU Limited, Unit 410 Cambridge Science Park, Milton Road, Cambridge, CB4 0PE, UK*

[b] [b]*Chirotech Technology Ltd, Dr Reddy's Laboratories, Steanard Lane, Mirfield, W. Yorks, WF14 8HZ, UK*

Contents:

Chromatographic Methods for Compounds **1**, **2a**, **2b**, **2c**, **3b**, **5**, **6**, **7**, **8**, **9**, **11** and **12**..... 2

Pilot Plant Procedures for 30 kg Manufacture of **1**..... 6

^{1}H NMR Spectra of Compounds **1**, **2a**, **2b**, **2c**, **3a**, **3b**, **9**, **11** and **12** ……..12

Chromatographic Methods for Compounds 1, 2a, 2b, 2c, 3b, 5, 6, 7, 8, 9, 11 and 12.

GC method for analysis of 4-(hydroxymethyl)benzaldehyde 5.

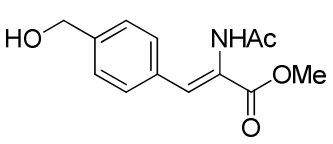
Column: Zebron ZB-5 30 m (0.25 mm × 0.25 μm).

Helium pressure: 20 psi.

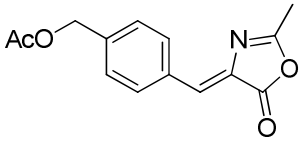
Oven program: 100 °C for 5 minutes, then 10 °C/minute for 12 minutes, then 220 °C for 3 minutes.

Retention times: terephthalic dialdehyde, 10.7 min; 4-hydroxymethyl-benzaldehdye (**5**) 13.2 min; 1,4-di-(hydroxymethyl)-benzene (**6**), 13.9 min.

HPLC method for analysis of methyl (*Z*)-2-(acetylamino)-3-[4-(hydroxymethyl) phenyl]propenoate 3b.



3b



9

Column: Apex ODS 5 μm (4.6 × 250 mm).

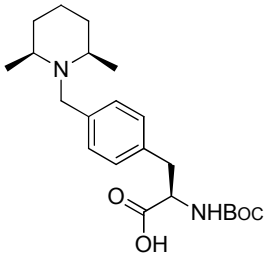
Mobile phase: Water : acetonitrile isocratic (60 : 40).

Detector: UV/Vis 214 nm.

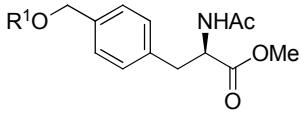
Temperature: 40 °C

Retention times: terephthaldialdehyde, 4.8 min; 4-hydroxymethyl(benzaldehyde) **5**, 3.4 min; 1,4-di-(hydroxymethyl)-benzene **6**, 2.8 min; (4-acetoxymethyl)benzaldehyde **7**, 6.1 min; 1,4-di-(acetoxymethyl)-benzene **8**, 8.8 min; azlactone **9**, 13.6 min; **3b**, 3.1 min.

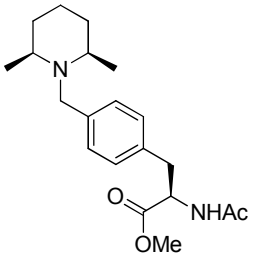
HPLC method for analysis of 1, 2b, 11 and 12.



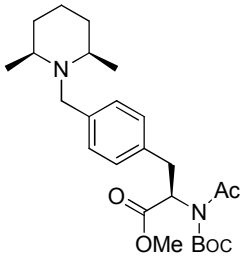
1



2b



11



12

Column: Kromasil C18 5 µm (4.6 × 150 mm)

Mobile phase: A: 10 mM KH_2PO_4 at pH 3.0 with H_3PO_4

B: Acetonitrile

Detection: UV at 220nm

Temperature: 40 °C.

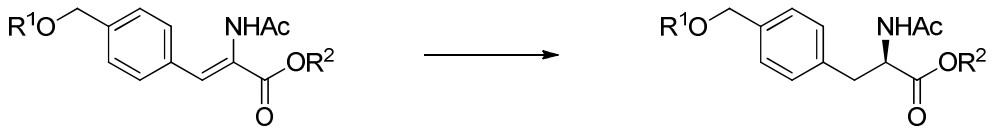
Run time: 35 minutes.

The gradient elution details are given in the table below;

Time	0	5	25	29	31	45
A	90	90	30	30	90	90
B	10	10	70	70	10	10

Retention times: **2b**, 10.8 min; **11**, 11.2 min, **12**, 18.7 min, **1**, 15.1 min.

HPLC method for chiral analysis of *N*-acetyl-4-(acetoxymethyl)-phenylalanine methyl ester 2a and *N*-acetyl-4-(hydroxmethyl)-phenylalanine methyl ester 2b.



3a R^1 = Ac, R^2 = Me
3b R^1 = H, R^2 = Me
3c R^1 = R^2 = H

2a R^1 = Ac, R^2 = Me
2b R^1 = H, R^2 = Me
2c R^1 = R^2 = H

Column: Chiracel OD (250 mm × 4.6 mm, 10 µm).

Flow rate: 1.0 ml min-1.

Mobile phase: Heptane: Propan-2-ol isocratic (80:20).

Detector: UV/Vis 254 nm.

Retention times: (*R*)-**2a**, 7.08 min; (*S*)-**2a**, 8.71 min; (*R*)-**2b**, 8.57; (*S*)-**2b**, 10.06 min.

HPLC method for chiral analysis of *N*-acetyl-4-(hydroxmethyl)-phenylalanine methyl ester 2b.

Column: Chiracel OJ (250 mm × 4.6 mm).

Flow rate: 1.0 ml min-1.

Mobile phase: Heptane: Propan-2-ol isocratic (85:15).

Detector: UV/Vis 214 nm.

Temperature: 40 °C.

Retention times: **3b**, 24.7 min; (*R*)-**2b**, 15.4; (*S*)-**2b**, 18.2 min.

HPLC method for chiral analysis of methyl (2*R*)-2-(acetylamino)-3-[4-(hydroxymethyl) phenyl]propanoic acid 2c.

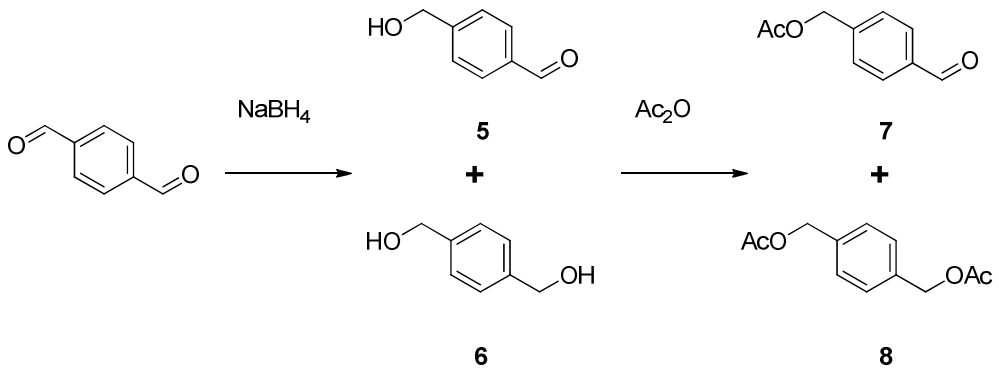
Column:	Chirobiotic T (250 mm × 4.6 mm, 5 μm).
Flow rate:	1ml/min
Detector:	UV/Vis 210 nm
Mobile phase:	Methanol:TEA:Acetic Acid (100:0.1:0.1).
Retention times:	(*S*), 5.80 min; (*R*), 10.78 min.

HPLC method for chiral analysis of 2(*R*)-(*tert*-butoxycarbonyl-amino)-3-(4-(2*R*,6*S*)-2,6-dimethylpiperidine-1-yl)-methyl-phenyl)-propionic acid 1.

Column:	Chirobiotic T (250 mm × 4.6 mm, 5 μm).
Flow rate:	0.8 ml min^{-1}.
Detector:	UV/Vis 210nm.
Mobile phase:	0.1% Triethylammonium acetate (pH 4.5): methanol isocratic (62:38).
Retention times:	(*S*), 10.77 min; (*R*), 14.68 min.

Pilot Plant Procedures for 30 kg Manufacture of 1

Preparation of 4-(Acetoxymethyl)benzaldehyde 7.

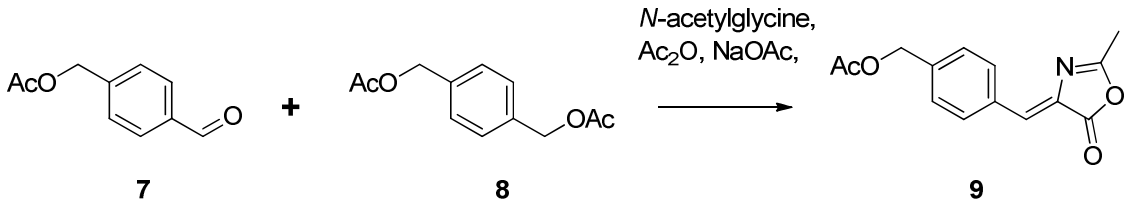


- To 250 litre reactor charge 32% sodium hydroxide (1.125 kg, 9 mol) and water (7.9 kg, mix).
- Charge sodium borohydride (2.7 kg, 71.4 mol) and dissolve.
- Transfer the borohydride solution to a drum.
- Charge terephthalaldehyde (27 kg, 201 mol) to 250 litre reactor.
- Purge and charge THF (71.3 kg)
- Add borohydride solution below 30 °C to reactor over 2 hours.
- Stir 1 hour and test.
- Charge water (40 kg) and toluene (39 kg).
- Transfer to 200L glass bowl and separate lower aqueous to waste.
- Transfer organics to reactor, charge water (40 kg) and stir for 15 min.
- Transfer to 200L glass bowl and separate lower aqueous to waste.
- Transfer organics to 500 litre reactor.

- Charge 3 batches of 4-(hydroxymethyl)benzaldehyde **5** solution to 500 litre reactor.
- Distil to remove THF and water (atmospheric to pot temperature of 90 °C.
- Heat to gentle reflux.
- Charge acetic anhydride (94.5 kg, 926 mol) over 2 hours.
- Maintain at reflux for 2 hours.
- Sample and test.
- Remove solvents under vacuum at 70 °C.

- Cool and drum up 4-(acetoxymethyl)benzaldehyde **7** product (110 kg, contains 73 kg of **7**).

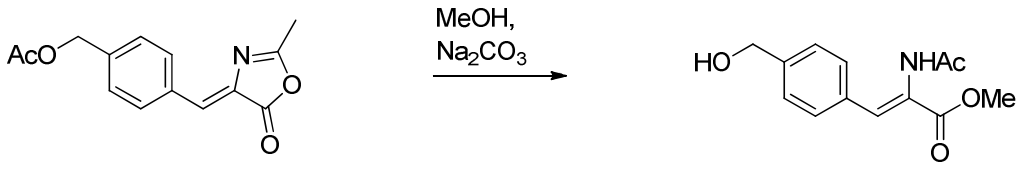
Preparation of 4-[(*Z*)-(2-methyl-5-oxo-1,3-oxazol-4(5*H*)-ylidene)methyl]benzyl acetate 9.



7 **8** **9**

- Charge sodium acetate (16.5 kg, 201 mol) and *N*-acetylglycine (23.3 kg, 199 mol) to 500 litre reactor.
- Purge and charge ethyl acetate (92.5 kg).
- Heat to 50-55 °C and charge acetic anhydride (41.2 kg, 403 mol) over 1 hour.
- Charge crude 4-(acetoxymethyl)benzaldehyde **7** (55 kg, ~205 mol) over 1 hour.
- Heat to reflux.
- Maintain at reflux for 20 hours, sample and test.
- Cool to 20-25 °C and charge water (70 kg) *via* dosing pump.
- Cool to 0-5 °C and filter.
- Wash with water
- Recharge wet cake to reactor and purge.
- Charge MTBE (76 kg) and stir at 0-5 °C for 30 mins.
- Filter and wash with MTBE (26 kg).
- Dry azlactone **9** (22.4 kg) under vacuum at 45 °C.

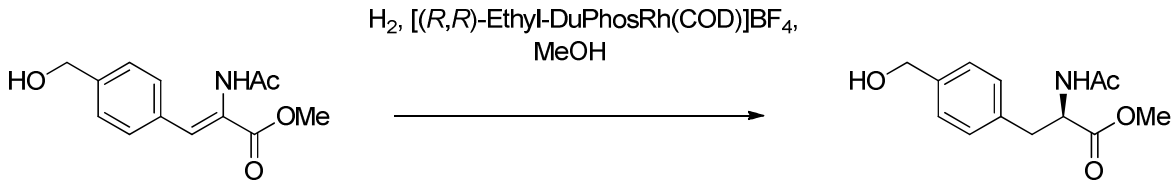
Preparation of methyl (*Z*)-2-(acetylamino)-3-[4-(hydroxymethyl)phenyl]propenoate 3b.



9 **3b**

- Charge azlactone **9** (44.7 kg, 181 mol) and sodium carbonate (0.05 kg, 0.47 mol) to 500 litre reactor.
- Charge methanol (265 kg) and stir for 4 hours.
- Sample and test.
- Discharge solution of **3b** via filter to drums.
- Wash through with methanol (40 kg).

Preparation of methyl (2*R*)-2-(acetylamino)-3-[4-(hydroxymethyl)phenyl]propanoate 2b.

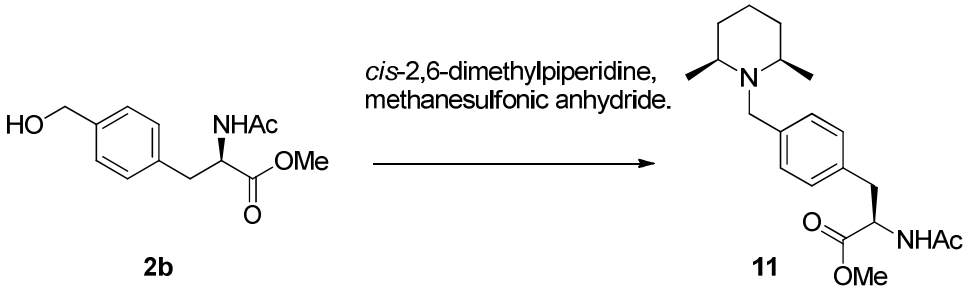


3b **2b**

- Charge solution of **3b** (175 kg, ~90.5 mol) to 300 litre hastelloy hydrogenator.
- Charge methanol line wash.
- Purge with nitrogen.
- Charge [(*R*,*R*)-Ethyl-DuPhosRh(COD)]BF_4 (19 g, 26 mmol) and purge.
- Hydrogenate at 100 PSI until uptake of gas ceases.
- Discharge to drums.
- Wash out vessel with methanol (20 kg) to drums.
- Charge solution to 500 litre reactor.
- Remove bulk of methanol/methyl acetate solvent under vacuum.
- Charge toluene (178 kg).
- Distil at atmospheric pressure to 110 °C.

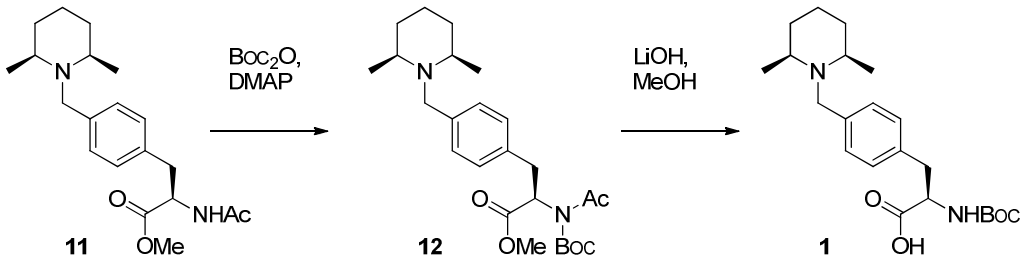
- Cool to <20 °C.
- Filter and wash with toluene (89 kg)
- Dry **2b** (43.3 kg) under vacuum at 45 °C.

Preparation of methyl (2*R*)-2-acetylamino-3-(4-{[(2*R*,6*S*)-2,6-dimethylpiperidin-1-yl]methyl}phenyl)propanoate 11.



- Charge **2b** (43.3 kg, 172 mol) and methanesulfonic anhydride (37.6 kg, 216 mol) to 500 litre reactor and purge.
- Charge dichloromethane (165 kg) and add *cis*-2,6-dimethylpiperidine (68.2 kg, 608 mol) maintaining the temperature below 30 °C.
- Stir for a further 1 hour and sample and test.
- Charge citric acid, 20% solution (185 kg) and extract.
- Separate the lower organics to waste.
- Charge dichloromethane (100 kg), stir 15 mins and separate.
- Run off the lower organics to waste.
- Charge 50% potassium carbonate solution (70 kg) slowly.
- Charge toluene (177 kg), stir 15 minutes and separate.
- Transfer the lower aqueous layer to drum.
- Transfer the organic layer to 200L glass bowl.
- Recharge aqueous and toluene, stir 15 mins and separate.
- Transfer the lower aqueous layer to waste.
- Recharge all the organics to the reactor.
- Vacuum distil at 70 °C to remove solvent.
- Drum up the product **11** liquid (50.7 kg).

2(*R*)-(*tert*-butoxycarbonyl-amino)-3-(4-(2*R*,6*S*)-2,6-dimethylpiperidine-1-yl)-methyl-phenyl)-propionic acid 1.

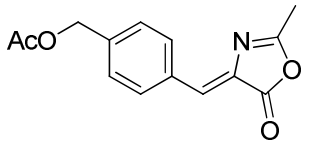


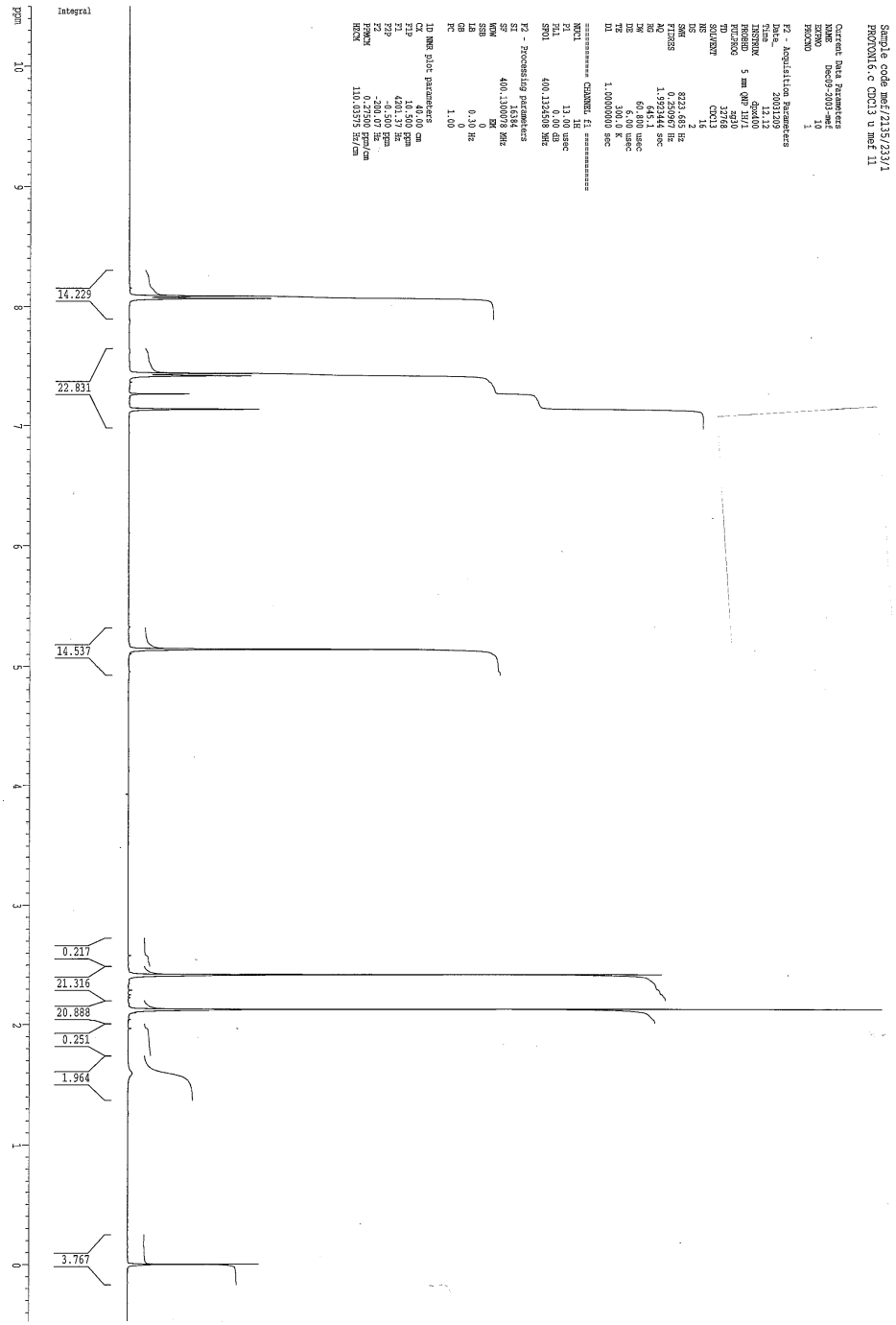
- Charge 4-dimethylaminopyridine (0.5 kg, 4.1 mol) and **11** (25.3 kg, 73.4 mol) to 500 litre reactor.
- Purge and charge THF (54 kg).
- Add di-*tert*-butyl dicarbonate (24.0 kg, 110 mol) via dosing pump, follow with THF (36 kg).
- Heat to 40 °C, and maintain for 24 hours, then sample and test.
- Charge water (10 kg) and cool to below 25 °C.
- Charge water (50 kg) and toluene (86.5 kg), stir for 15 minutes and allow to separate.
- Discharge the lower aqueous layer and wash the organics twice with water.
- Distil under vacuum at 50 °C to give **12** as a dark oil.
- Cool to below 0-5 °C and charge THF (72 kg).
- Charge a solution of lithium hydroxide monohydrate (6.12 kg, 146 mol) in water (50 kg)/methanol (16 kg) *via* the dosing pump over 75 minutes, maintain the temperature at 0-5 °C.
- Stir for 75 minutes, sample and test.
- Adjust the pH to 6.0 with 25% potassium hydrogensulfate (100 kg), maintain the temperature below 15 °C.
- Distil at 50 °C to remove THF and methanol.
- Wash the aqueous layer with MTBE (30 kg).
- Extract the product into dichloromethane (×2, 110 kg and 55 kg).
- Charge water (100 kg) and distil under vacuum at 50 °C to remove dichloromethane.
- Charge Amberlite IRA-67 (9.2 kg) and adjust the pH to 6.0-6.5.
- Screen to remove the resin.

- Distil under vacuum at 50 °C to remove the bulk of the water.
- Charge ethanol (40 kg) and distil under vacuum at 50 °C to remove the bulk of the solvent.
- Charge ethyl acetate (45 kg) and distil under vacuum at 50 °C to remove the bulk of the solvent.
- Charge ethyl acetate (37 kg) and stir for 30 minutes.
- Charge MTBE (62 kg) and cool to below 25 °C and maintain.
- Cool to 0-5 °C and maintain for 2 hours.
- Filter and wash with a mixture of ethyl acetate (14 kg) and MTBE (23 kg).
- Dry product **1** under vacuum at 45 °C to remove the bulk of the solvent.
- Dry under vacuum at 70 °C to remove the bulk of the solvent, yield 20 kg, 52.8 mol.

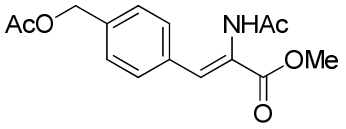
^{1}H NMR Spectra of Compounds 1, 2a, 2b, 2c, 3a, 3b, 3c, 9, 11 and 12.

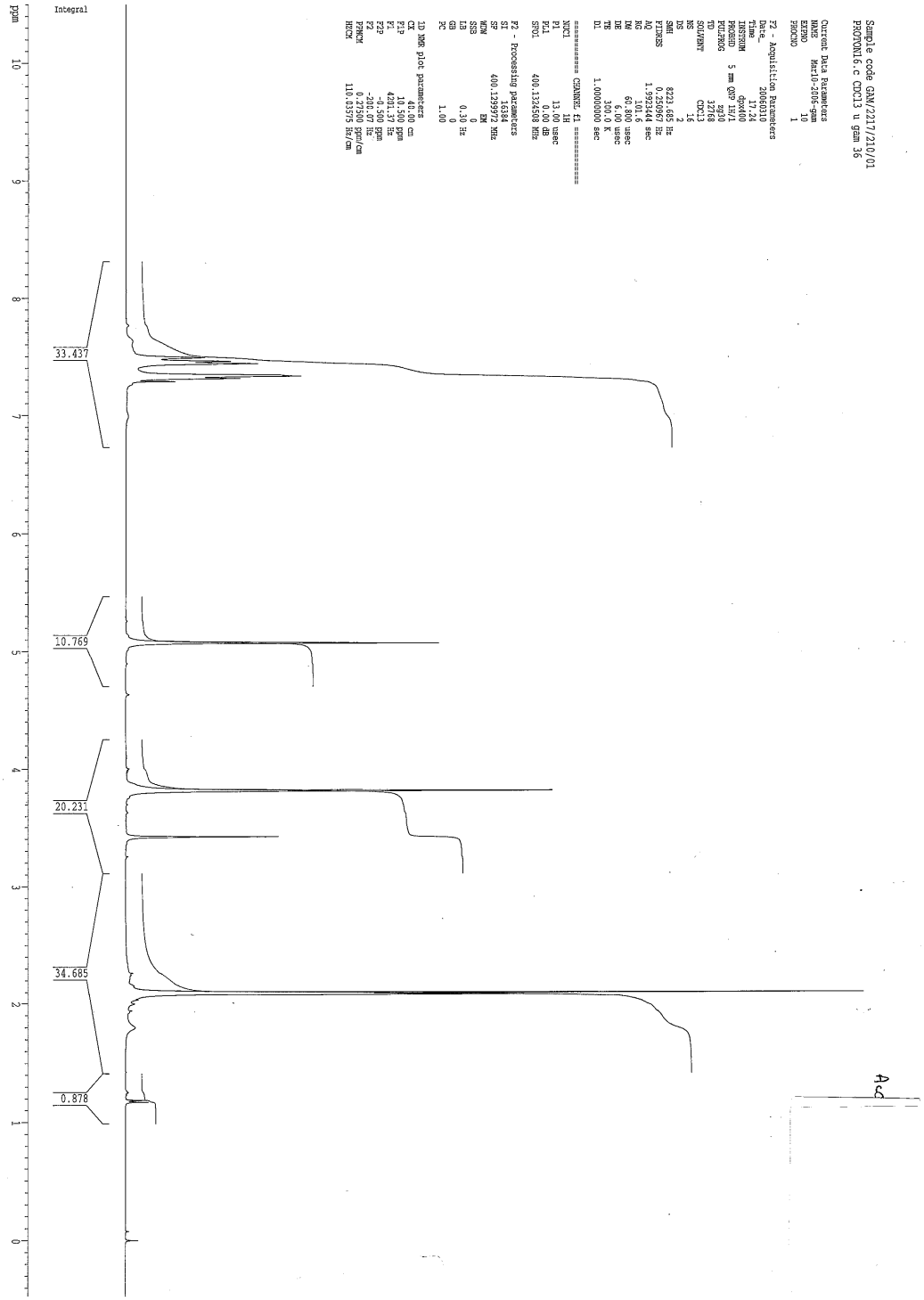
4-[(*Z*)-(2-Methyl-5-oxo-1,3-oxazol-4(5*H*)-ylidene)methyl]benzyl acetate 9 ($CDCl_3$, 400 MHz).



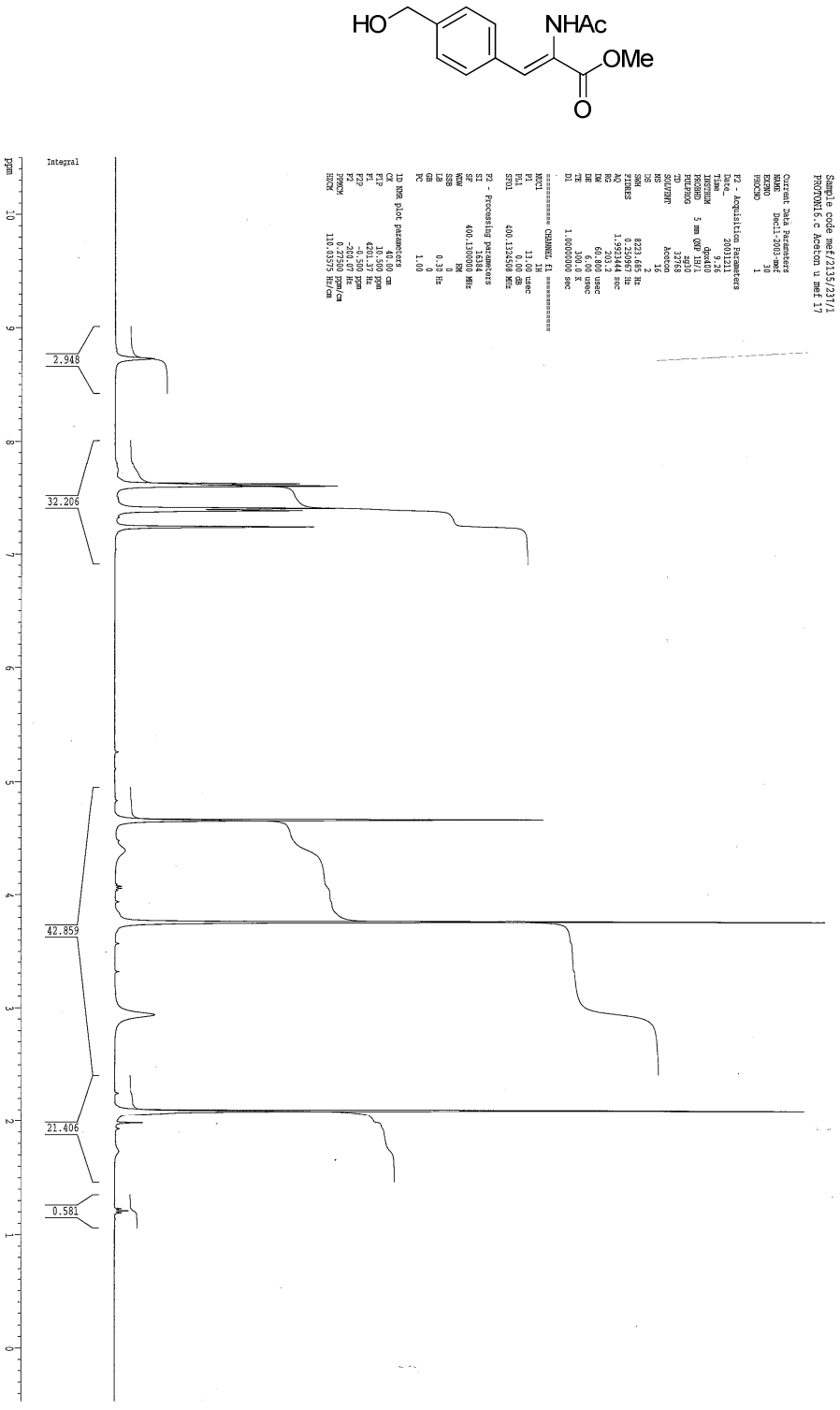


Methyl (*Z*)-2-(acetylamino)-3-[4-(acetoxymethyl)phenyl]propenoate) 3a (400 MHz, $CDCl_3$).

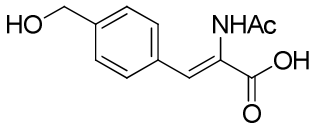


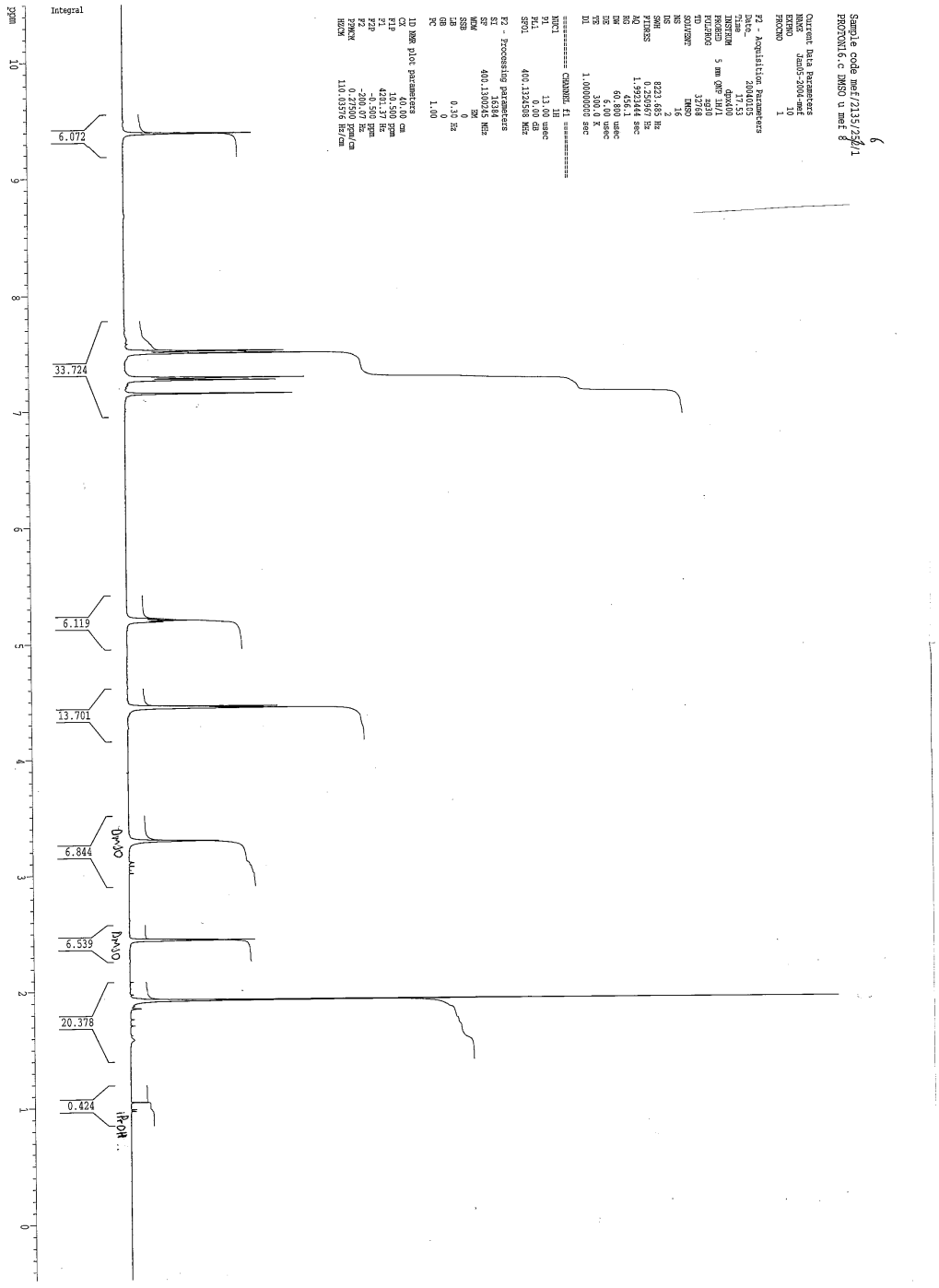


Methyl (*Z*)-2-(acetylamino)-3-[4-(hydroxymethyl)phenyl]propenoate 3b. (400 MHz, acetone-d6).

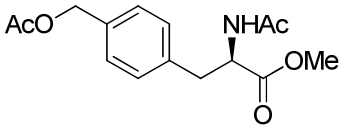


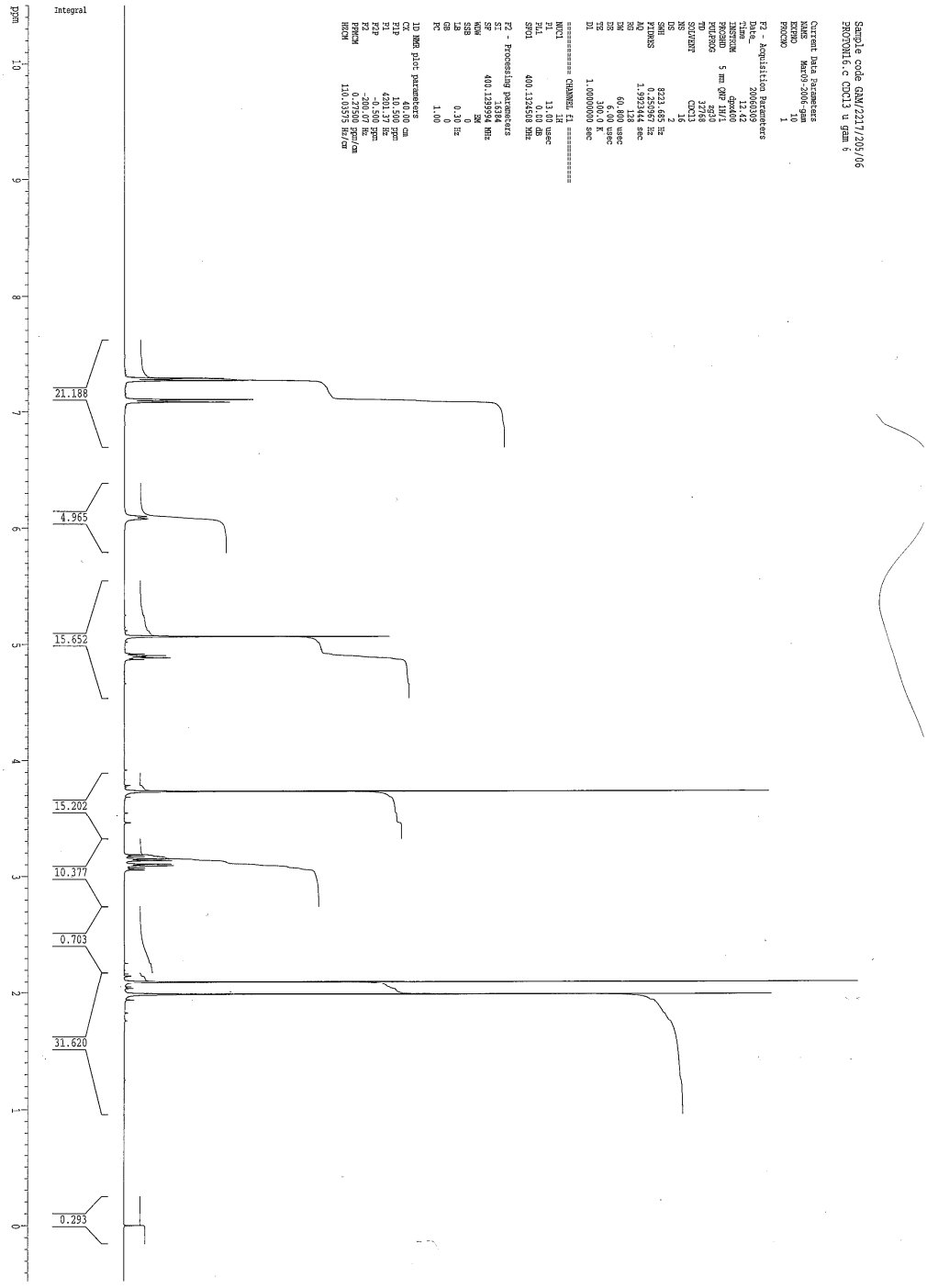
(*Z*)-2-(Acetylamino)-3-[4-(hydroxymethyl)phenyl]propenoic acid 3c (400 MHz, DMSO-d6).



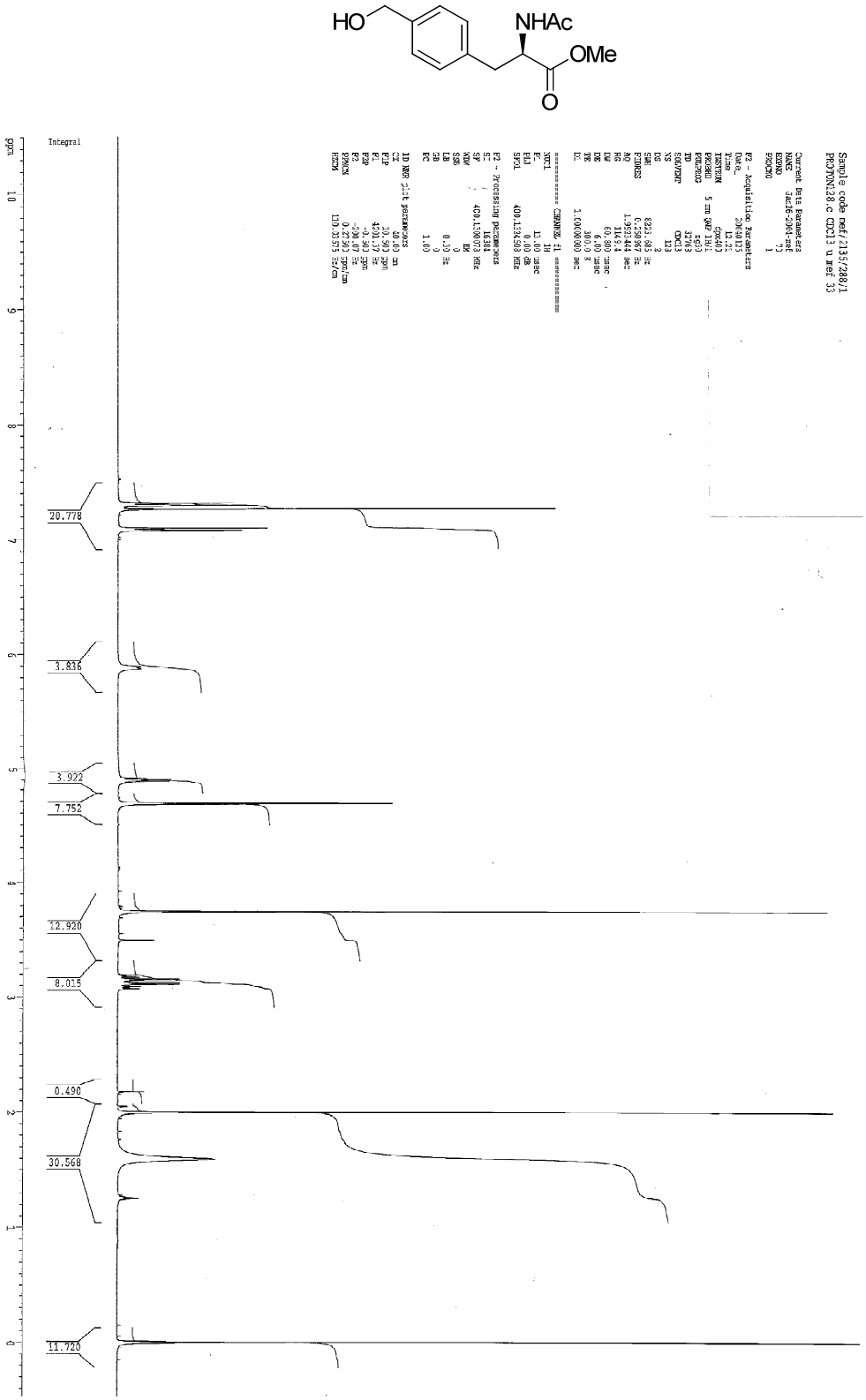


Methyl (2*R*)-2-(acetylamino)-3-[4-(acetoxymethyl)phenyl]propanoate 2a (400 MHz, $CDCl_3$).

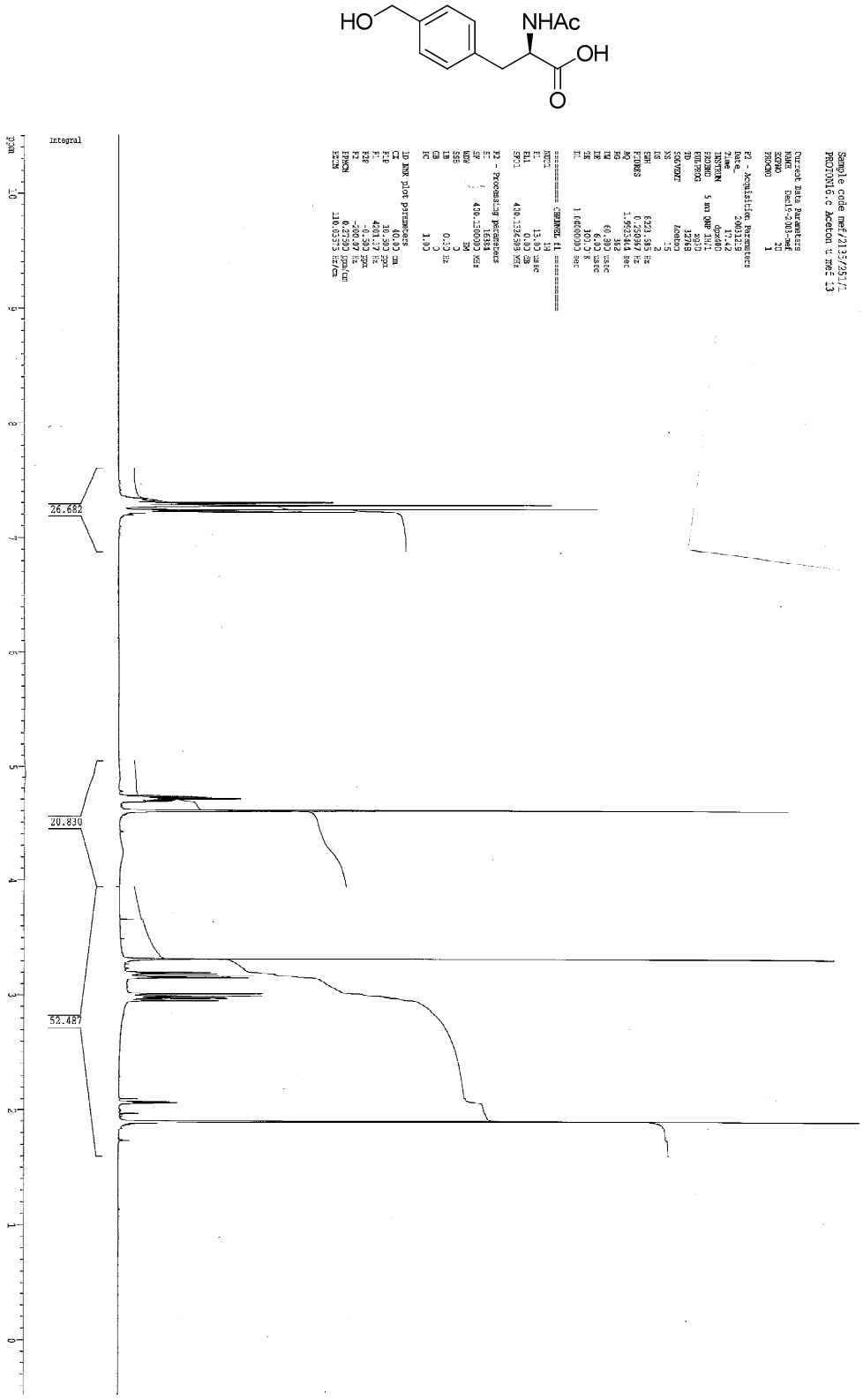




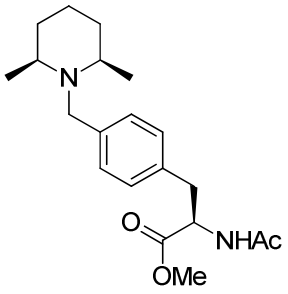
Methyl (2*R*)-2-(acetylamino)-3-[4-(hydroxymethyl)phenyl]propanoate 2b ($CDCl_3$, 400 MHz).

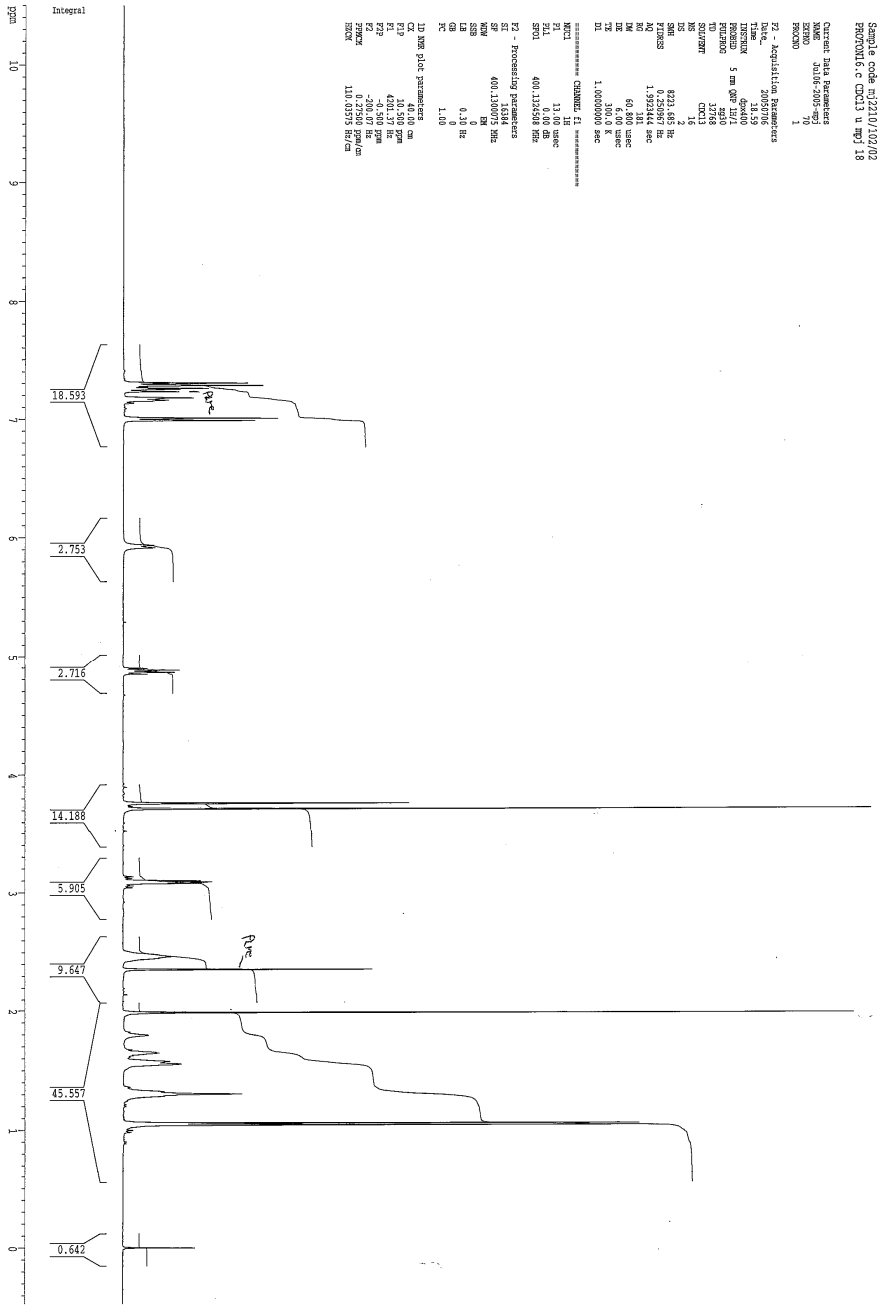


(2*R*)-2-(Acetylamino)-3-[4-(hydroxymethyl)phenyl]propanoic acid 2c (acetone-d6, 400 MHz).

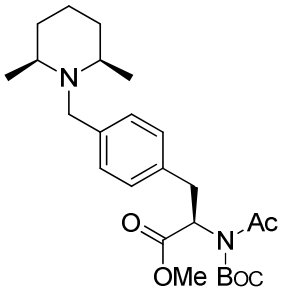


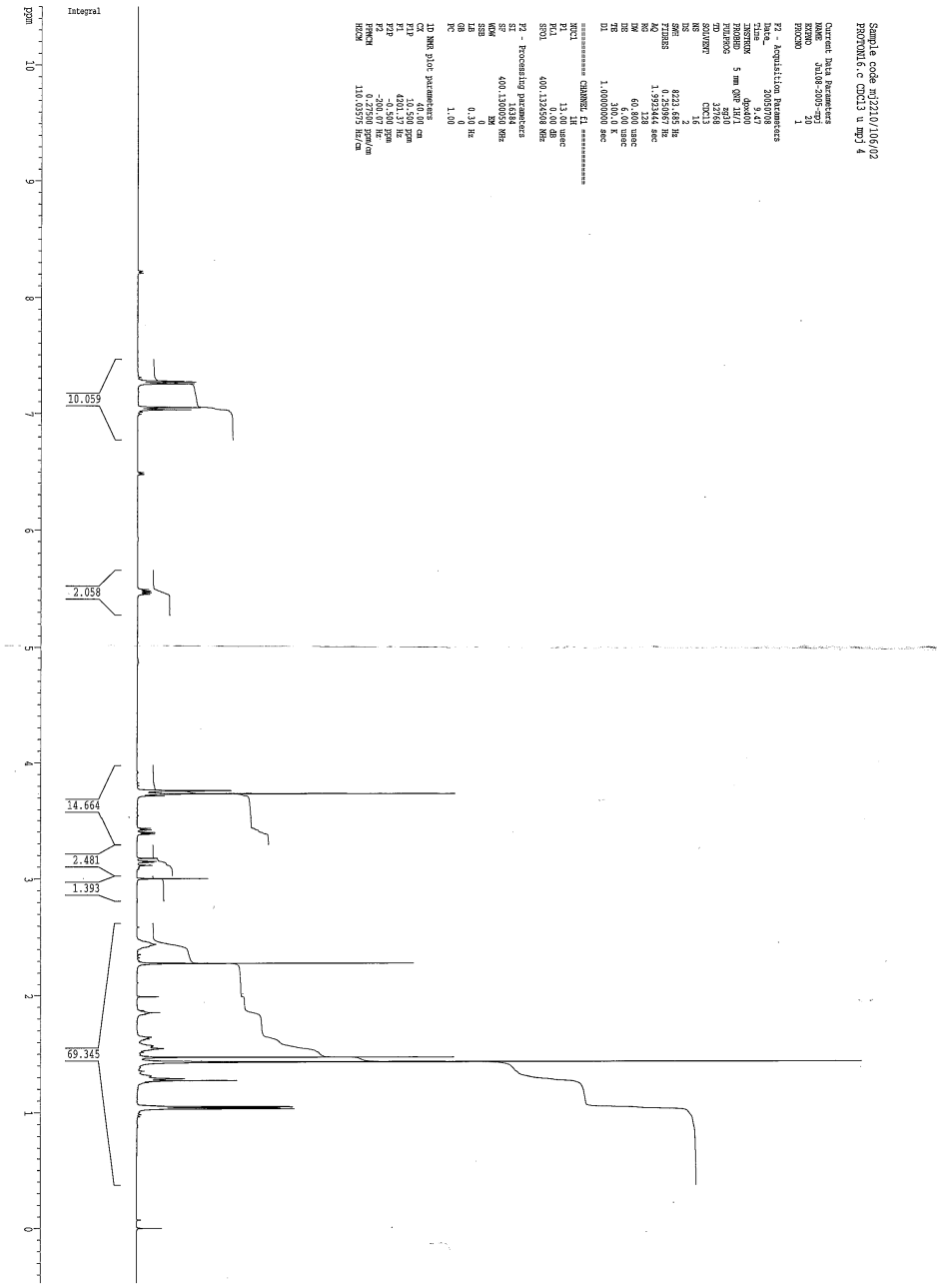
Methyl (2*R*)-2-acetylamino-3-(4-{[(2*R*,6*S*)-2,6-dimethylpiperidin-1-yl]methyl}phenyl) propanoate 11 (400 MHz, $CDCl_3$).





Methyl 2(*R*)-acetyl-(*tert*-butoxycarbonyl)-amino-3-(4-{[(2*R*,6*S*)-2,6-dimethylpiperidin-1-yl]-methyl}phenyl)propanoate 12 (400 MHz, $CDCl_3$).





2(*R*)-(*tert*-butoxycarbonyl-amino)-3-(4-(2*R*,6*S*)-2,6-dimethylpiperidine-1-yl)-methyl-phenyl)-propionic acid 1 (400 MHz, DMSO).