Enzymatic cascade flow synthesis of protected mandelonitrile derivatives

Mariëlle M. E. Delville,[a] Kaspar Koch,[b] Jan C. M. van Hest,[a] Floris P. J. T. Rutjes* [a]

[a] *Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands*

[b] *FutureChemistry B.V., Toernooiveld 100, 6525 EC Nijmegen, The Netherlands*

Supplementary Information

Chemicals

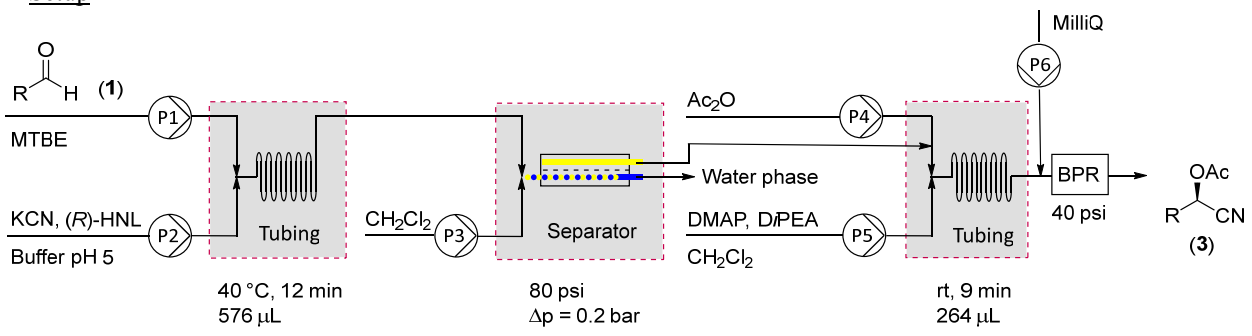
Benzaldehyde and furfural were freshly distilled before use. Ac_2O was purified over P_2O_5, filtered and neutralized with K_2CO_3. After a second filtration pure Ac_2O was obtained after distillation, stored under Ar(g). (*R*)-HNL was kindly provided by DSM Research. The wild type gene encoding for (*R*)-HNL, originating from bitter almonds (*Prunus amygdalus*), was cloned and efficiently expressed in the yeast strain *Pichia pastoris*. The enzyme was secreted from the cells (*Pichia pastoris*), and was obtained from the cell free supernatant by concentration using ultrafiltration/diafiltration.[1] Other chemicals were used as obtained from commercial sources.

Technical background information

The syringe pumps of the model NE-1000 were used. Tubing of fluorinated ethylene propylene (FEP) bought from Upchurch Scientific was used to connect the syringes to the reactor (also made out of from FEP-tubing) using connectors PEEK (polyether ether ketone) material. The backpressure regulator also bought from Upchurch Scientific (P-455) , made from PEEK material with a maximum capacity of 1000 psi was used holding a cartridge of 40 psi (P-761). The separator used was bought from Syrris model FRX FLLEX.

General enzymatic flow procedure for acetyl-protected cyanohydrins 3 (Table 4)

Setup



1 Glieder, A.; Weis, R.; Skranc, W.; Poechlauer, P.; Dreveny, I.; Majer, S.; Wubbolts, M.; Schwab, H.; Guber, K. *Angew. Chem. Int. Ed.* **2003**, *42*, 4815-4818.

The reaction was performed in FEP-tubing of 576 μL (ID = 0.75 and 0.5 mm). Temperature was maintained at 40 °C by the use of a heated water bath. After 12 min of residence time, the reaction mixture was diluted with CH_2Cl_2 at rt before separating the phases using the FRX FLLEX module from Syrris. The back pressure of the FLLEX was set to 80 psi to prevent back flushing. A pressure difference of 0.2 bar was set over the two channels. The water phase was collected and the organic phase was directly sent to the second reaction. The second reaction was carried out in FEP-tubing of 264 μL (ID = 0.75 mm). After 9 min the reaction mixture was diluted with MilliQ to solubilize the formed salts before flowing through a back pressure regulator (BPR) of 40 psi.

Solutions

Solution A: desired aldehyde (0.69 mmol) in 3 mL MTBE.

Solution B: solid KCN (269 mg, 4.14 mmol) and solid citric acid (542 mg, 2.58 mmol) combined before adding 5.4 mL MilliQ. Once everything was dissolved 600 μL of (*R*)-HNL lysate was added.

Solution C: neat Ac_2O (10.6 M).

Solution D: DMAP (1.1 mmol, 147 mg) dissolved in 2 mL CH_2Cl_2 before adding 2 mL D*i*PEA.

Experimental

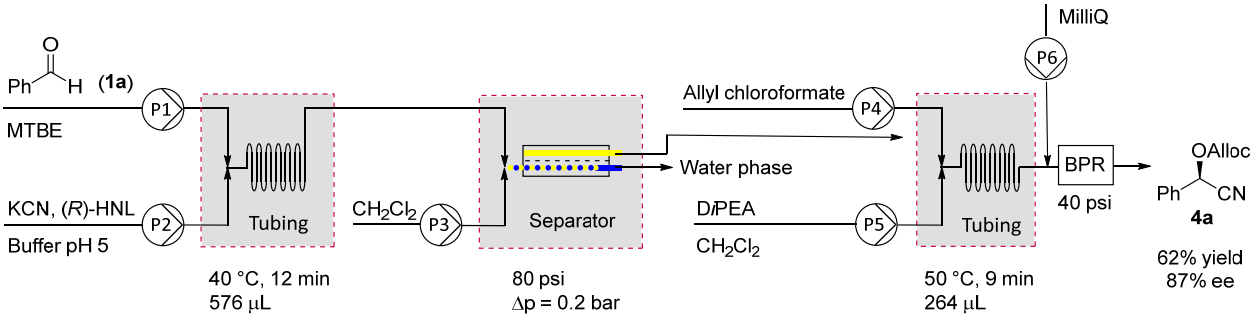
Solution A (8 μL/min) and solution B (40 μL/min) were combined. After 12 min of residence time the reaction mixture was diluted with CH_2Cl_2 (10 μL/min) at rt before separating the phases using the FLLEX. The water phase was collected and the organic layer was directly sent to the second reaction. First solution C was added (4 μL/min), directly followed by solution D (8 μL/min). After 9 min the reaction mixture was diluted with MilliQ (30 μL/min). The obtained crude product was purified by extraction with CH_2Cl_2 (3 × 3mL). The combined organic layers were dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was further purified using flash column chromatography (silica, heptane/ethyl acetate 96:4). The fractions containing product were concentrated under reduced pressure to determine the isolated yield. Products were further characterized by ^{1}H NMR spectroscopy and chiral HPLC analysis.

General batch procedure for the synthesis of racemic acetylated compounds

KCN (2 mmol, 130 mg, 4 equiv.) was dissolved in 250 μL MilliQ before adding the desired aldehyde (0.5 mmol, 1 equiv.). This was directly followed by the addition of 250 μL AcOH. After stirring for 2 hours (sometimes adding additional portions of KCN to help the reaction reach complete conversion, as determined by TLC) the reaction mixture was diluted with 5 mL H_2O and extracted with Et_2O (3* 3mL). The combined organic layers were dried over Na_2SO_4, filtrated and concentrated under reduced pressure. To the residue 2 mL toluene was added, followed by evaporation of the solvent. The residue was then dissolved in 1 mL dry CH_2Cl_2 followed by the addition of Ac_2O and DiPEA. The reaction was stirred overnight before diluting with 5 mL H_2O and extraction with 3 mL Et_2O. The organic layer was washed with 5 mL H_2O. The combined organic layers were again extracted with 3 mL Et_2O. The first and second organic layers were combined, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was further purified using flash column chromatography (silica, heptane) using an eluent of heptane: ethyl acetate 96:4. The fractions containing product were concentrated under reduced pressure, followed by analysis with ^{1}H NMR and chiral HPLC.

Enzymatic flow procedure for Alloc-protected (*R*)-mandelonitrile (4a, Table 5, entry 1)

Setup



The reaction was performed in FEP-tubing of 576 µL (ID = 0.75 and 0.5 mm). Temperature was maintained at 40 °C by the use of a heated water bath. After 12 min of residence time the reaction mixture was diluted with CH_2Cl_2 at rt before separating the phases using the FRX FLLEX module from Syrris. The back pressure of the FLLEX was set to 80 psi to prevent back flushing. A pressure difference of 0.2 bars was set over the two channels. The water phase was collected and the organic phase was directly sent to the second reaction. The second reaction was carried out in FEP-tubing of 264 µL (ID = 0.75 mm). Temperature of the second reaction was maintained at 50 °C by the use of a heated water bath. After 9 min the reaction mixture was diluted with MilliQ to solubilize the formed salts before flowing through a back pressure regulator (BPR) of 40 psi.

Solutions

Solution A: benzaldehyde (0.69 mmol) in 3 mL MTBE.
Solution B: solid KCN (269 mg, 4.14 mmol) and solid citric acid (542 mg, 2.58 mmol) combined before adding 5.4 mL MilliQ. Once everything was dissolved 600 µL of (*R*)-HNL lysate was added.
Solution C: neat allyl chloroformate (9.4 M).
Solution D: neat D*i*PEA (5.7 M).

Experimental

Solution A (8 µL/min) and solution B (40 µL/min) were combined. After 12 min of residence time the reaction mixture was diluted with CH_2Cl_2 (4 µL/min) at rt before separating the phases using the FLLEX module. The water phase was collected and the organic phase was directly sent to the second reaction. First solution C was added (4 µL/min) directly followed by solution D (8 µL/min). After 9 min the reaction mixture was diluted with MilliQ (30 µL/min). The set-up was stabilized for 1 hour and 10 min. Product was collected for 1 hour and 20 min. The obtained crude product was purified by extraction with Et_2O (3 × 3 mL). The combined organic layers were dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was further purified using flash column chromatography (silica, heptane/ethyl acetate 96:4) followed by a second flash column (silica, CH_2Cl_2). The fractions containing product were concentrated under reduced pressure to obtain the product (20 mg, 62% yield and 87% ee). Analysis was performed with ^{1}H NMR, ^{13}C NMR, HRMS and chiral HPLC.

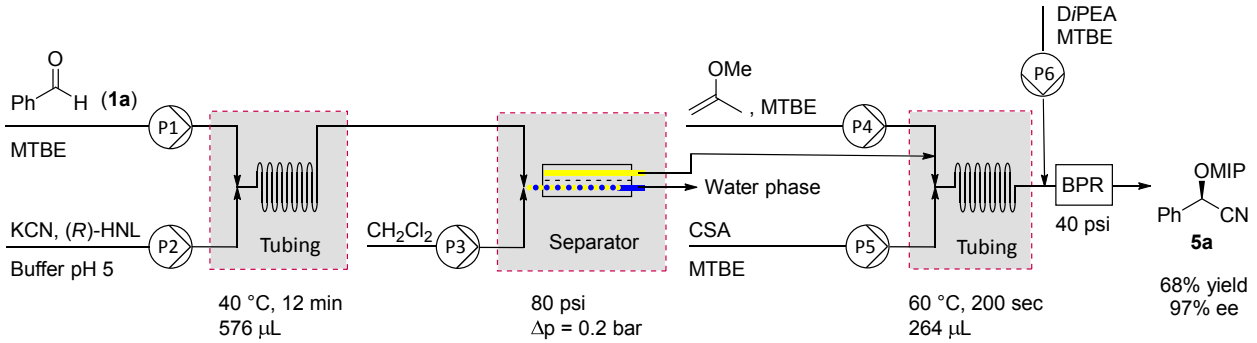
Recycling of water phase

From the previous experiment 5 mL of water phase was collected: solution E. Solution A (8 µL/min) and solution E (40 µL/min) were combined. After 12 min of residence time the reaction mixture was diluted with CH_2Cl_2 (4 µL/min) at rt before separating the phases using the FLLEX module. The water phase was collected and the organic phase was directly sent to the second reaction. First solution C was added (4 µL/min) directly followed by solution D (8 µL/min). Temperature was maintained at 50 °C by the use of a heated water bath. After 9 min the reaction mixture was diluted with MilliQ (30 µL/min) before flowing through a back pressure regulator (BPR) of 40 psi. The set-up was stabilized for 50 min. Product was collected for 45 min. The obtained crude product was purified by extraction with Et_2O (3 × 3 mL). The combined organic layers were dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was further purified using flash column chromatography (silica, CH_2Cl_2), followed by a second flash column (silica, CH_2Cl_2). A third flash column was needed (silica, heptane/ethyl acetate 96:4), as was a

fourth flash column (silica, CH_2Cl_2). The fractions containing product were concentrated under reduced pressure to obtain the product (9.4 mg, 52% yield, and 80% ee). ^{1}H NMR and chiral HPLC analysis were performed.

Enzymatic flow procedure for MIP-protected (*R*)-mandelonitrile (5a, Table 5, entry 2)

Setup



The reaction was performed in FEP-tubing of 576 µL (ID = 0.75 and 0.5 mm). Temperature was maintained at 40 °C by the use of a heated water bath. After 12 min of residence time the reaction mixture was diluted with CH_2Cl_2 at rt before separating the phases using the FRX FLLEX module from Syrris. The back pressure of the FLLEX was set to 80 psi to prevent back flushing. A pressure difference of 0.2 bars was set over the two channels. The water phase was collected and the organic phase was directly sent to the second reaction. The reaction was carried out in FEP-tubing of 264 µL (ID = 0.75 mm). Temperature of the second reaction was maintained at 60 °C by the use of a heated water bath. After 200 sec, the reaction mixture was quenched with D*i*PEA to finalize the synthesis. A back pressure regulator (BPR) of 40 psi was used to keep the reaction in the liquid phase.

Solutions

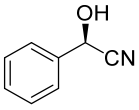
Solution A: desired aldehyde (0.69 mmol) in 3 mL MTBE.
Solution B: solid KCN (269 mg, 4.14 mmol) and solid citric acid (542 mg, 2.58 mmol) combined before adding 5.4 mL MilliQ. Once everything was dissolved 600 µL (*R*)-HNL enzyme was added.
Solution C: 2-methoxypropene (523 µl, 8.1 mmol) in 2.5 mL MTBE
Solution D: camphorsulfonic acid (1.6 mg, 0.0072 mmol) in 3 mL MTBE
Solution Q: D*i*PEA (146 µL, 0.84) in 3 mL MTBE

Experimental

Solution A (8 µL/min) and solution B (40 µL/min) were combined. After 12 min of residence time the reaction mixture was diluted with CH_2Cl_2 (4 µL/min) at rt before separating the phases using the FLLEX module. The water phase was collected and the organic phase was directly sent to the second reaction. First solution C was added (12 µL/min) directly followed by solution D (11 µL/min). After 200 sec the reaction mixture was quenched with D*i*PEA (11 µL/min). The set-up was stabilized for 45 min. Product was collected for 1 hour and 50 min. The obtained crude product was purified by extraction with Et_2O (1* 3mL). The organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The product was obtained (28 mg, 68% yield and 97% ee). ^{1}H NMR and chiral HPLC analysis were performed.

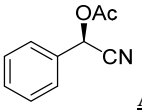
Compound characterization

NMR spectra were acquired at ambient temperature with a Bruker DMX 300 MHz spectrometer (^{1}H 300 MHz, ^{13}C 75 MHz). Carbon-13 spectra were proton-decoupled. ^{1}H NMR spectra were referenced to TMS or to the residual solvent peak. ^{13}C NMR spectra were referenced to the residual solvent peak. Electron ionization (EI) mass spectrometry was carried out using a JEOL AccuTOF-GCv. The AccuTOF-GCv consited of an Agilent 7890A GC, G4513A, HP-5MS column coupled to a JMS-100GCv system program. Chiral HPLC (cHPLC) was performed using a Shimadzu LC-2010C.



(*R*)-Mandelonitrile (**2a**, table 1)

A calibration curve for chiral HPLC was prepared with anisole as internal standard. cHPLC: Diacel AD-H column, 215nm, eluent heptane:isopropanol 90:10, elution time 7.0 min (*S*) and 7.9 min (*R*). The *R*-enantiomer was bought from Sigma-Aldrich as reference.

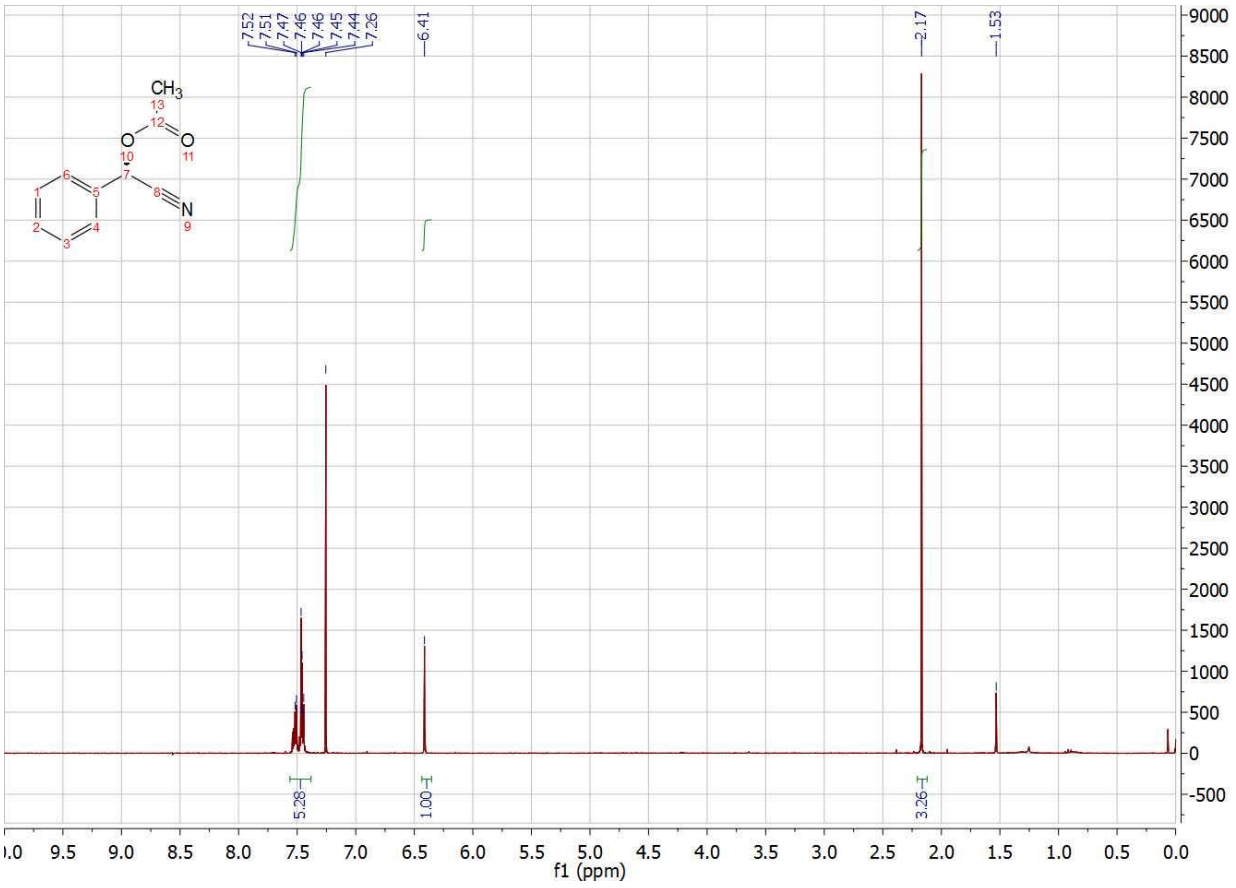


Acetyl protected (*R*)-mandelonitrile (**3a**, Table 3)

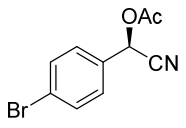
^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.52-7.44 (m, 5H, Ar H), 6.41 (s,1H, CH), 2.17 (s, 3H, Ac). ^{1}H NMR spectrum is in accordance with literature.[2]

cHPLC: Reprosil OM Dr Maisch column, 215nm, eluent heptane:isopropanol 99:1, elution time 13 min (*R*) and 16 min (*S*).

^{1}H NMR Acetyl protected (*R*)-mandelonitrile (**3a**)



2 Kadam, S. T.; Kim, S. S. *Tetrahedron* **2009**, *65*, 6330-6334.

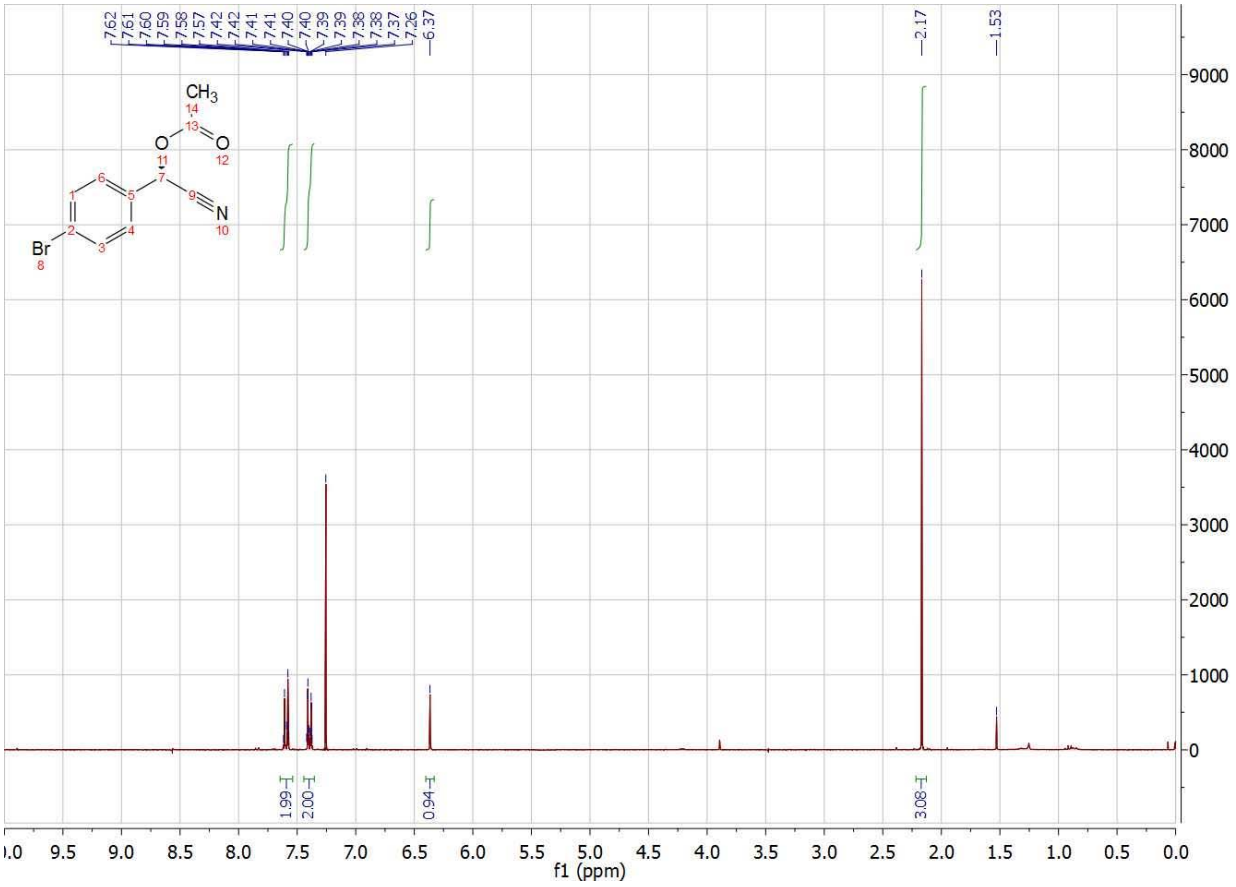


Acetyl protected (*R*)-4-bromomandelonitrile (**3b**, Table 4, entry 1)

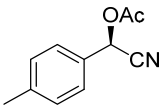
^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.65-7.45 (m, 2H, Ar H), 7.44-7.35 (m, 2H, Ar H), 6.37 (s, 1H, CH), 2.17 (s, 3H, Ac). ^{1}H NMR spectrum is in accordance with literature.[3]

cHPLC: Reprosil OM Dr Maisch column, 215nm, eluent heptane:isopropanol 99:1, elution time 21 min (*R*) and 26 min (*S*).

^{1}H NMR Acetyl protected (*R*)-4-bromomandelonitrile (**3b**)



3 Hatano, M.; Ikeno, T.; Miyamoto, T.; Ishiharam K. *J. Am. Chem. Soc.* **2005**, *127*, 10776-10777.

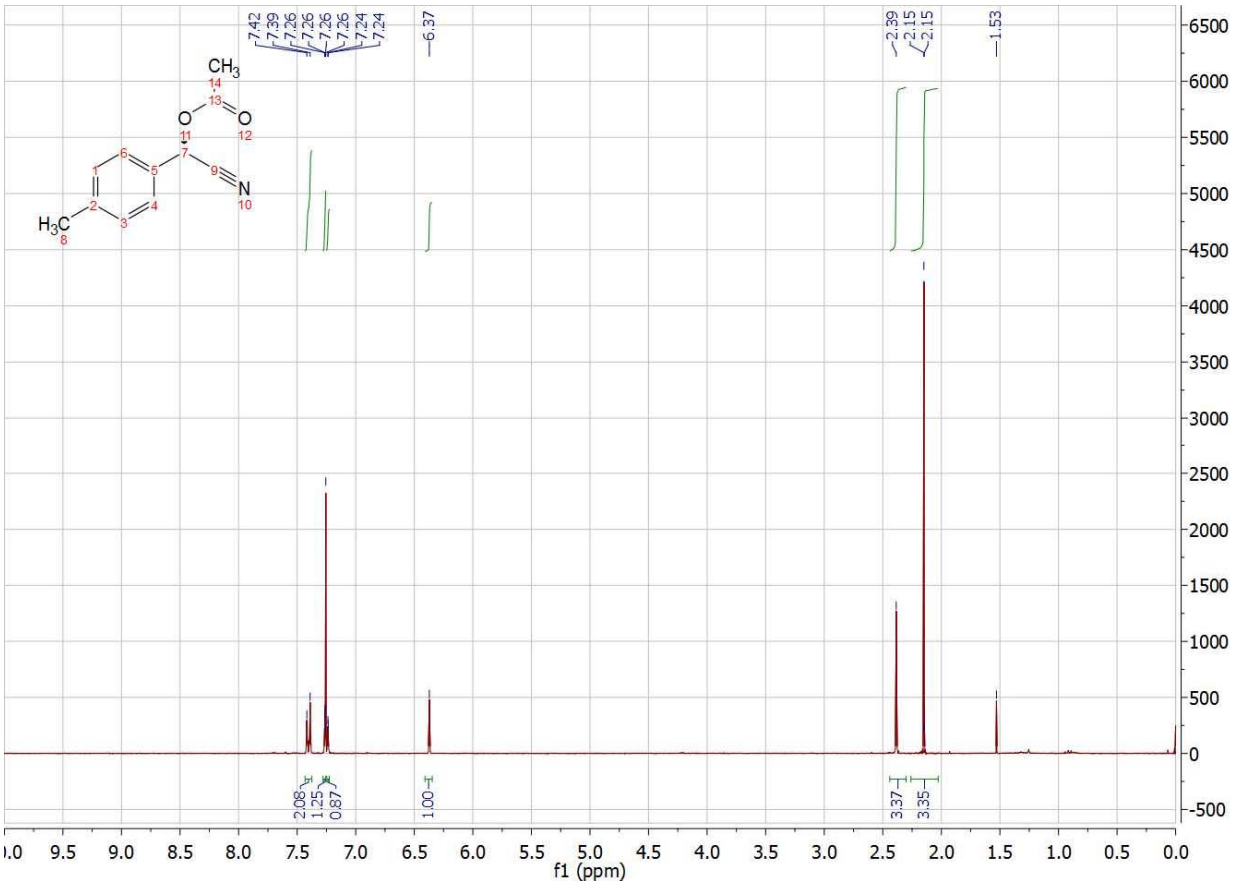


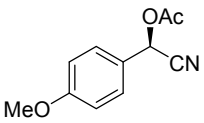
Acetyl protected (*R*)-4-methylmandelonitrile (**3c**, Table 4, entry 2)

^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.40 (d, *J* = 8.2 Hz, 2H, Ar H), 7.25 (d, *J* = 8.2 Hz, 2H, Ar H), 6.37 (s, 1H, CH), 2.39 (s, 3H, CH_3), 2.15 (s, 3H, Ac). ^{1}H NMR spectrum is in accordance with literature.[2,4]

cHPLC: Reprosil OM Dr Maisch column, 215nm, eluent heptane:isopropanol 90:10, elution time 5.9 min (*R*) and 7.0 min (*S*).

^{1}H NMR Acetyl protected (*R*)-4-methylmandelonitrile (**3c**)



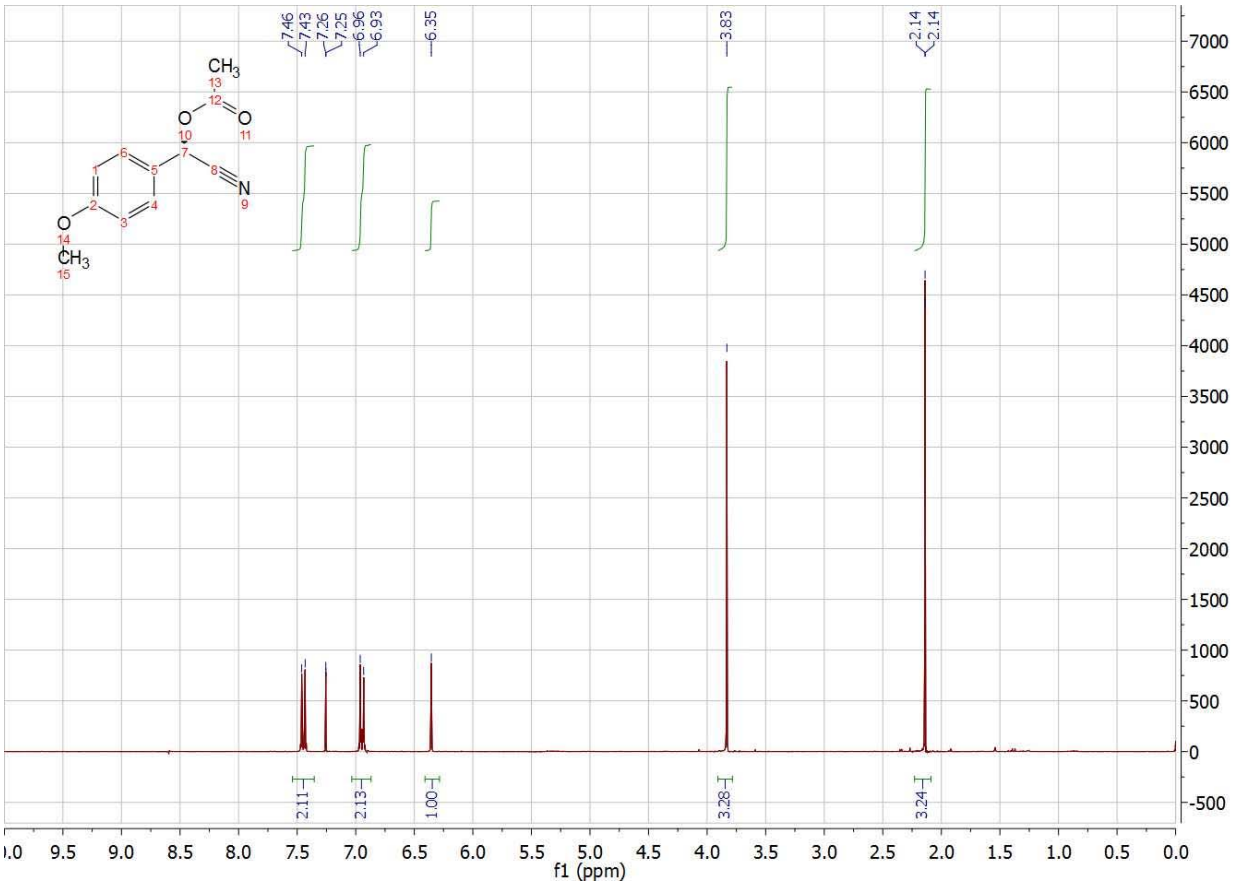


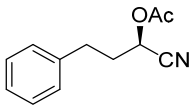
Acetyl protected (*R*)-4-methoxymandelonitrile (**3d**, Table 4, entry 3)

^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.45 (d, *J* = 8.6 Hz, 2H, Ar H), 6.95 (d, *J* = 8.9 Hz, 2H, Ar H), 6.35 (s, 1H, CH), 3.83 (s, 3H, $-OCH_3$), 2.14 (s, 3H, Ac). ^{1}H NMR spectrum is in accordance with literature.2 Note: 7.92 ppm should be 6.92 ppm.

cHPLC: Reprosil OM Dr Maisch column, 215nm, eluent heptane:isopropanol 99:1, elution time 20 min (*R*) and 23.5 min (*S*).

^{1}H NMR Acetyl protected (*R*)-4-methoxymandelonitrile (**3d**)



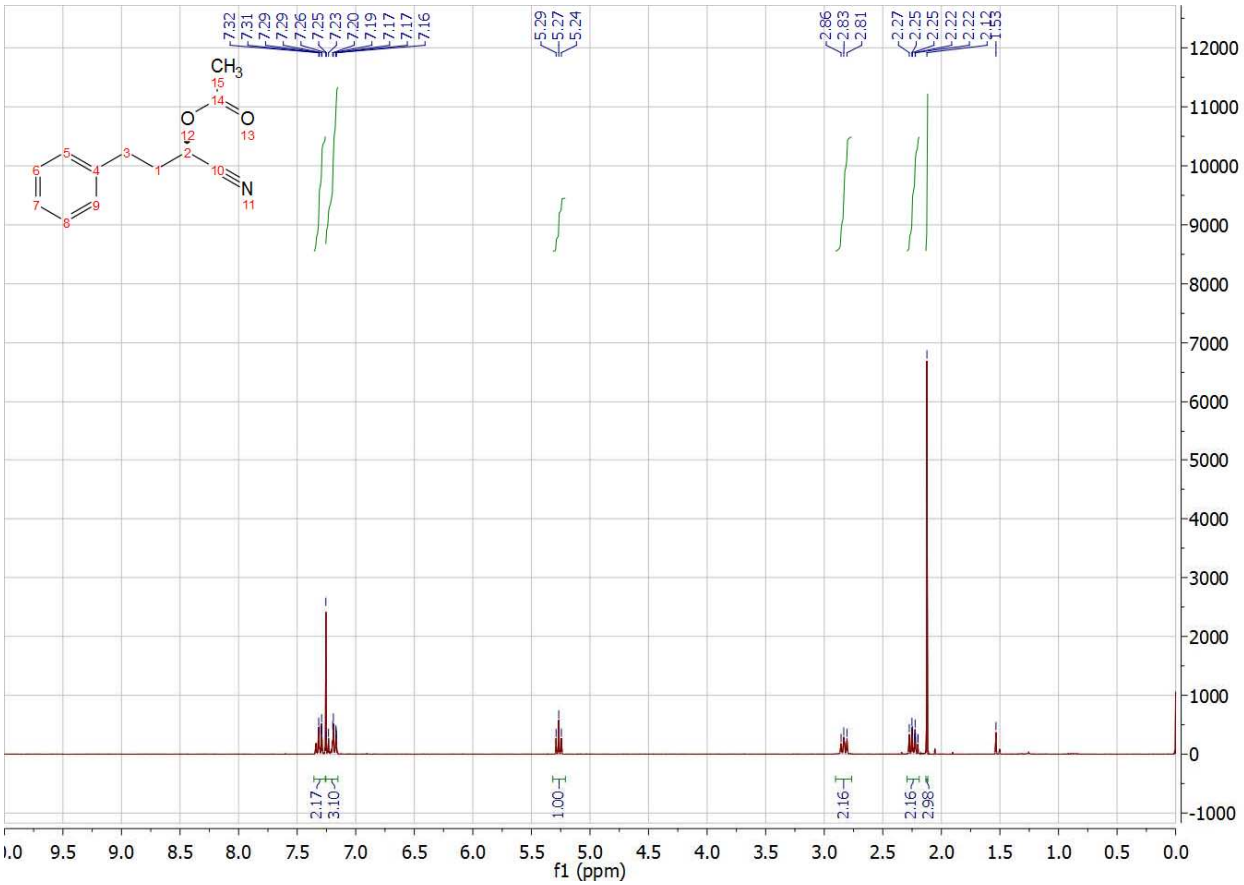


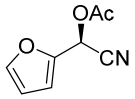
(*R*)-1-Cyano-3-phenylpropylacetate (**3e**, Table 4, entry 4)

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.32-7.16 (m, 5H, Ar H), 5.27 (t, *J* = 6.8 Hz, 1H, CH), 2.87-2.78 (m, 2H, CH_2), 2.29-2.91 (m, 2H, CH_2), 2.12 (s, 3H, Ac). ^{1}H NMR spectrum is in accordance with literature.2

cHPLC: Reprosil OM Dr Maisch column, 215nm, eluent heptane:isopropanol 95:5, elution time 15 min (*R*) and 17 min (*S*).

^{1}H NMR (*R*)-1-Cyano-3-phenylpropylacetate (**3e**)

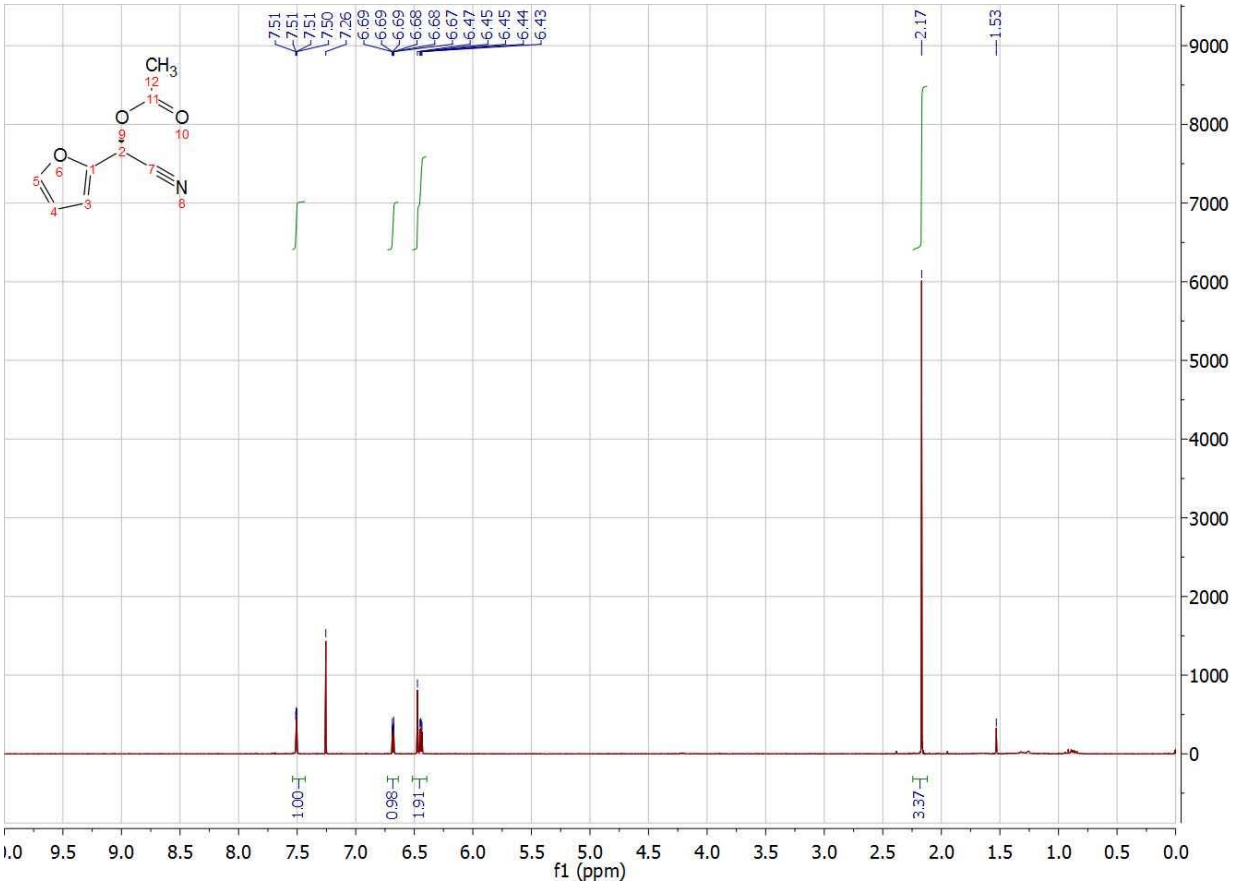




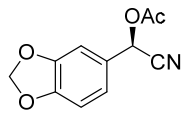
(*S*)-Cyano(furan2-yl)methyl acetate (**3f**, Table 4, entry 5)

^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.52 (dd, *J* = 1.9, 0.8 Hz, 1H, Ar H), 6.69 (dd, *J* = 2.3, 1.6 Hz, 1H, Ar H), 6.48 (s, 1H, Ar H), 6.45 (dd, *J* = 3.4, 1.8 Hz, 1H, CH), 2.17 (s, 3H, Ac). ^{1}H NMR spectrum is in accordance with literature.[4] cHPLC: Diacel AD-H column, 215nm, eluent heptane:isopropanol 95:5, elution time 8 min (*R*) and 9 min (*S*).

^{1}H NMR (*S*)-Cyano(furan2-yl)methyl acetate (**3f**)



4 Sakai, T.; Wang, K.; Ema, T. *Tetrahedron* **2008**, *64*, 2178-2183.

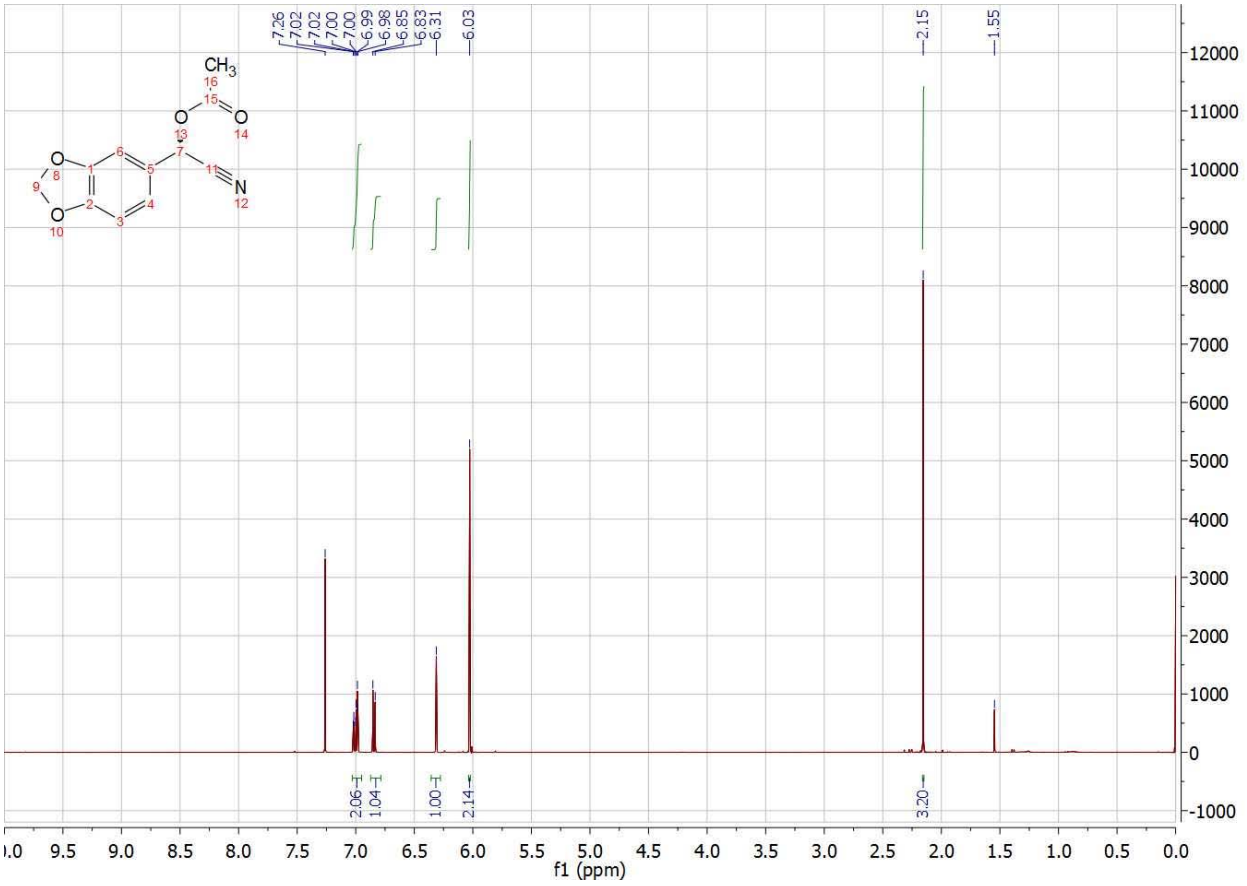


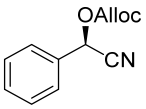
(*R*)-Benzo[d][1,3]dioxol-5-yl(cyano)methyl acetate (**3g**, Table 4, entry 6)

^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.03-6.97 (m, 2H, Ar H), 6.84 (d, *J* = 8.0 Hz, 1H, Ar H), 6.31 (s, 1H, CH), 6.03 (s, 2H, CH_2), 2.15 (s, 3H, Ac). ^{1}H NMR spectrum is in accordance with literature.3

cHPLC: Reprosil OM Dr Maisch column, 215nm, eluent heptane:isopropanol 99:1, elution time 27 min (*R*) and 31 min (*S*).

^{1}H NMR (*R*)-Benzo[d][1,3]dioxol-5-yl(cyano)methyl acetate (**3g**)





Alloc-protected mandelonitrile (**4a**, Table 5, entry 1)

^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.59-7.51 (m, 2H, Ar H), 7.51-7.42 (m, 3H, Ar H), 6.27 (s, 1H, CH), 5.93 (ddt, *J* = 17.2, 10.4, 5.9 Hz, 1H, CH), 5.36 (ddq, *J* = 24.9, 10.4, 1.3 Hz, 2H, CH_2), 4.76 - 4.66 (m, 2H, CH_2).

^{13}C NMR (75 MHz, $CDCl_3$, δ): 153.5 (C=O), 131.3, 130.8, 130.7, 129.5, 128.1, 120.2, 115.8, 70.0, 66.7.

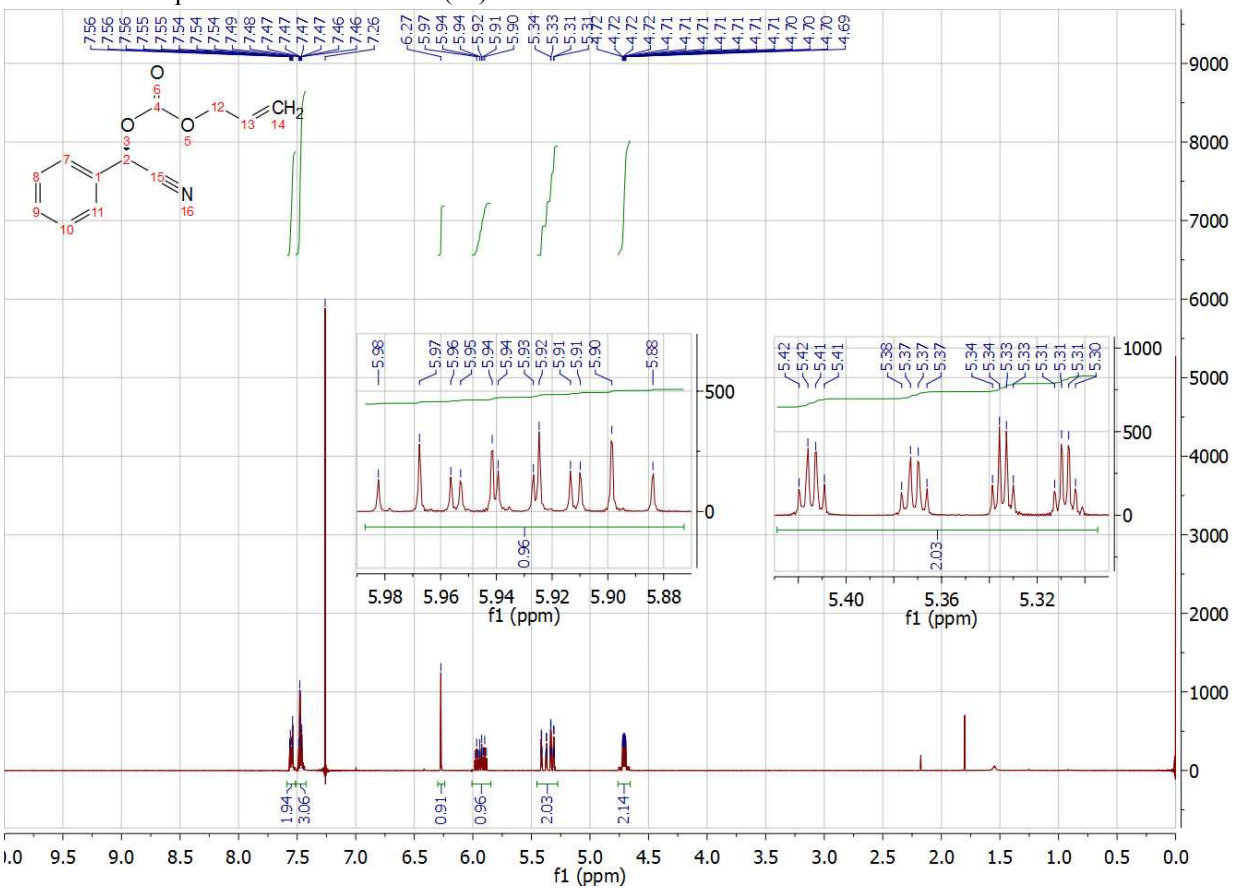
IR (neat, cm $^{-1}$): 3069, 3038, 2956, 1753, 1458, 1368, 1234, 943, 914, 784, 764, 695.

HRMS (EI$^+$) (*m/z*): $[M+H]^+$ calcd for $C_{12}H_{11}NO_3$, 217.074; found 217.074.

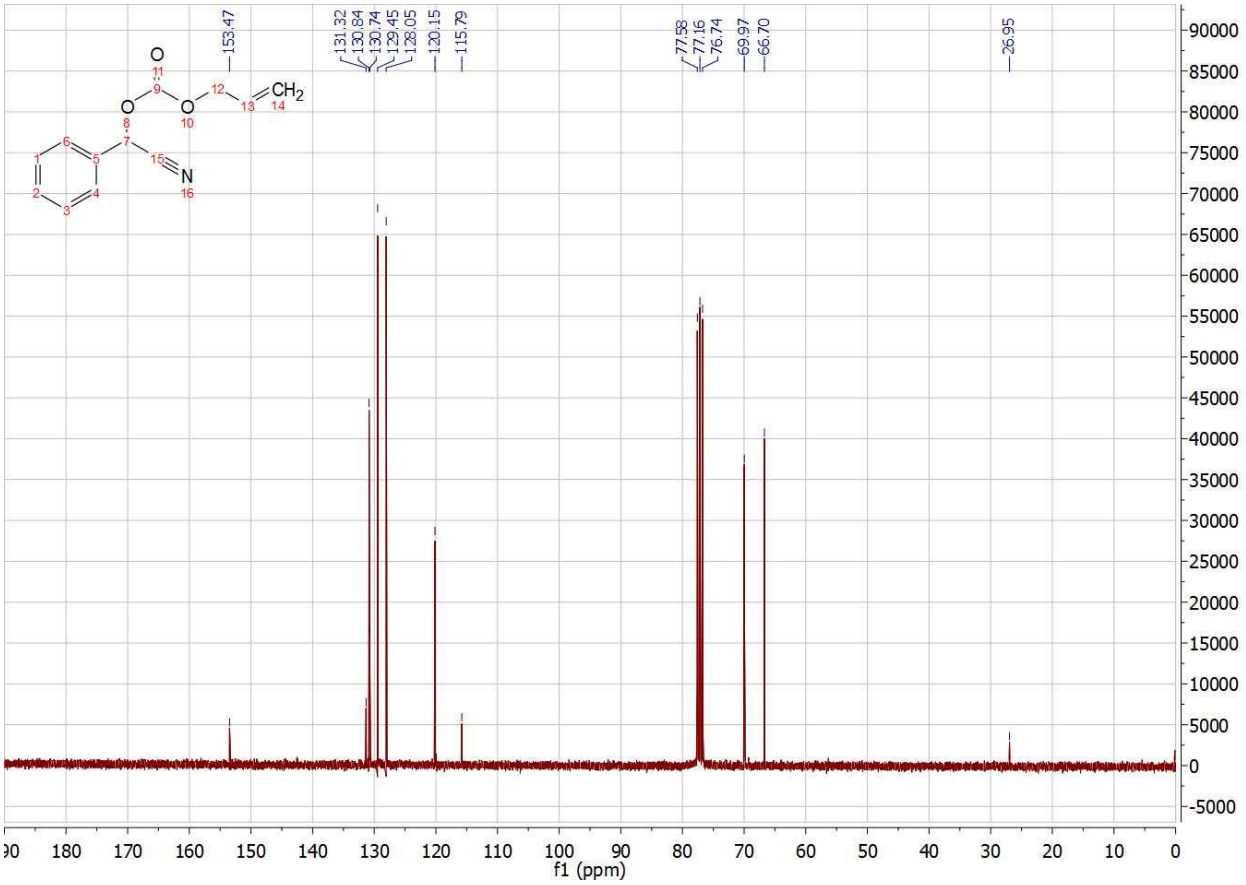
$[\alpha]^{25}_D$: + 16.4 (c = 0.28, CH_2Cl_2).

cHPLC: Reprosil OM Dr Maisch column, 215nm, eluent heptane:isopropanol 90:10, elution time 6.2 min (*R*) and 7.0 min (*S*).

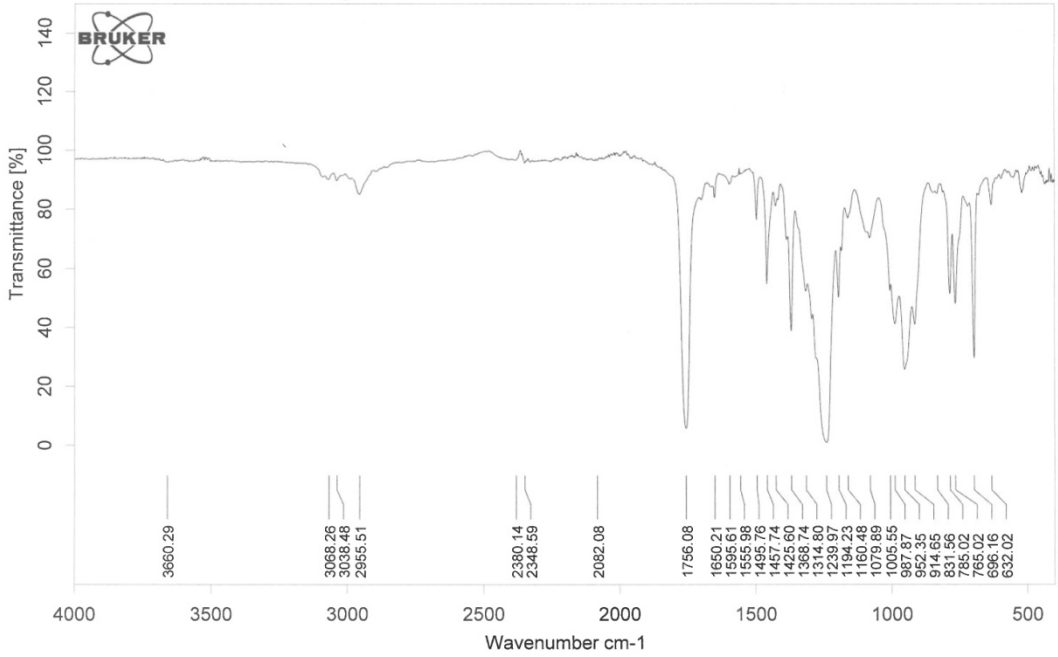
^{1}H NMR Alloc-protected mandelonitrile (**4a**)



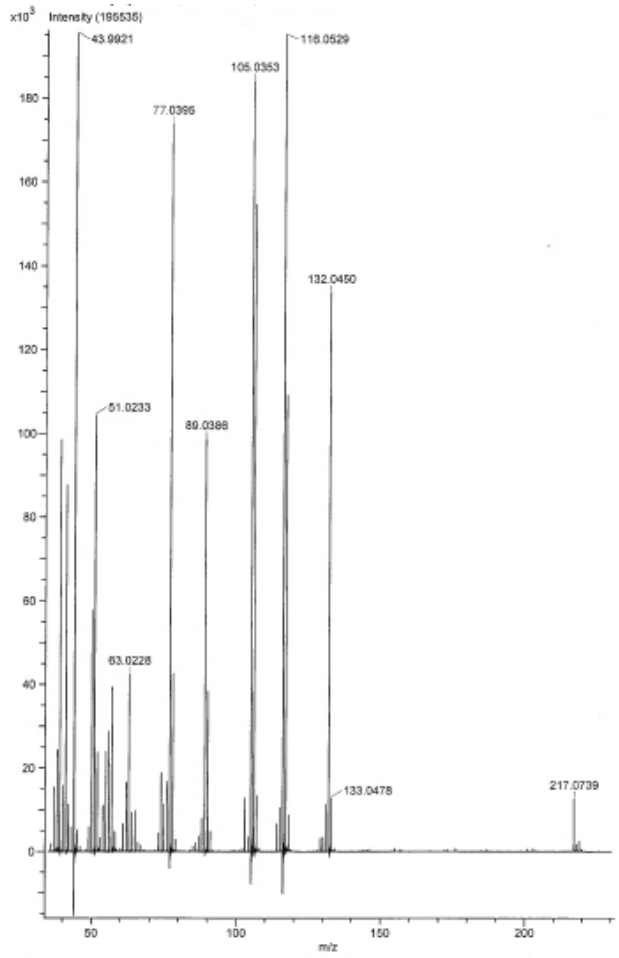
^{13}C NMR Alloc-protected mandelonitrile (**4a**)



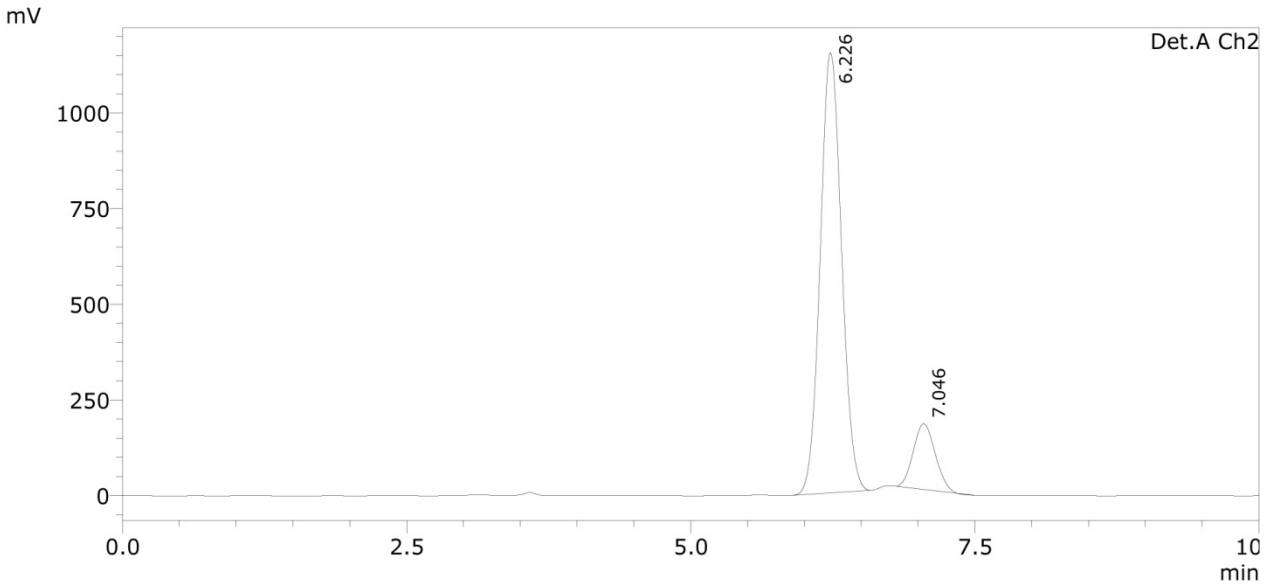
IR Alloc-protected mandelonitrile (**4a**)

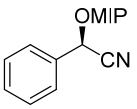


HRMS Alloc-protected mandelonitrile (**4a**)



cHPLC Alloc-protected mandelonitrile (**4a**)



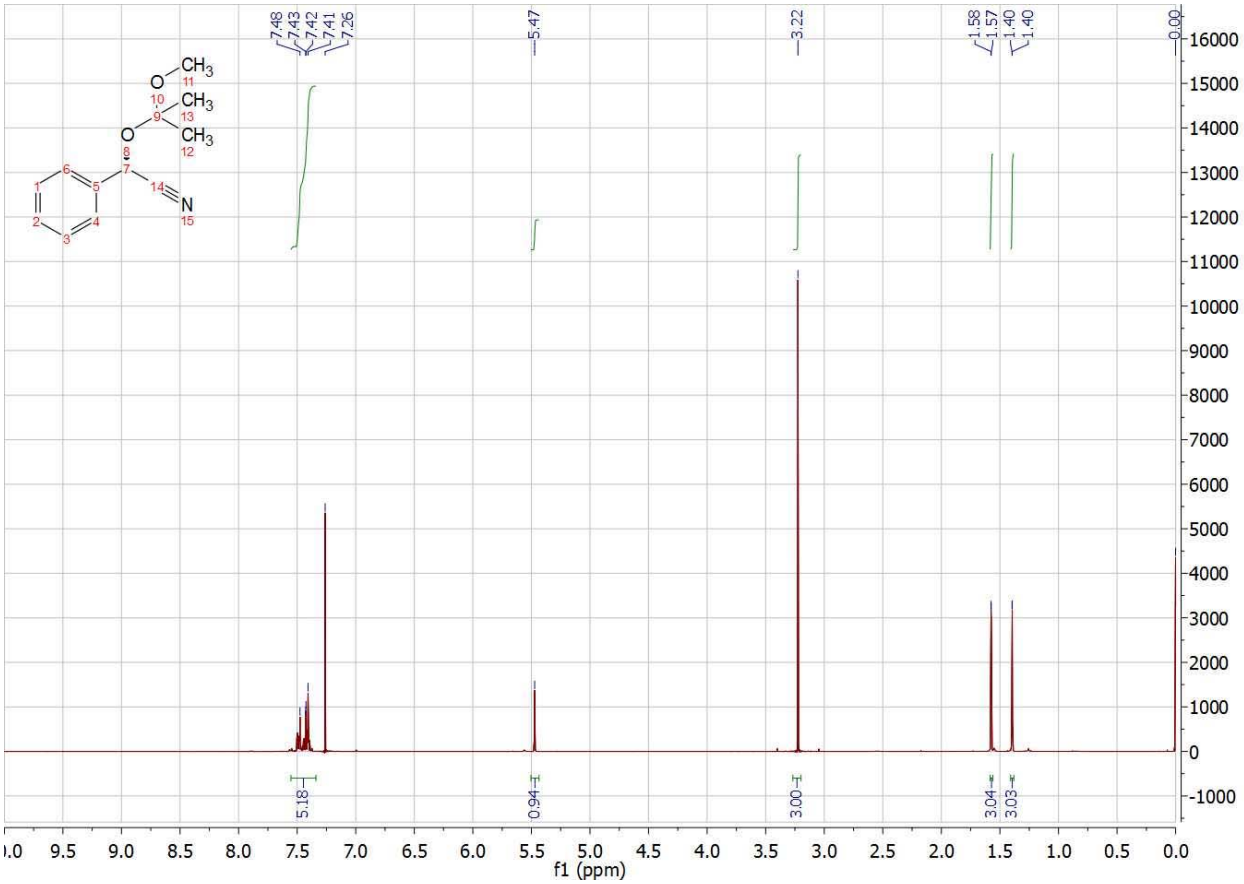


MIP-protected (*R*)-mandelonitrile (**5a**, Table 5, entry 2)

^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.55-7.34 (m, 5H, Ar H), 5.47 (s, 1H, CH), 3.22 (s, 3H, -OCH_3), 1.58 (d, *J* = 0.6 Hz, 3H, CH_3) , 1.40 (d, *J* = 0.6 Hz, 3H, CH_3). ^{1}H NMR spectrum is in accordance with literature.[5]

cHPLC: Reprosil OM Dr Maisch column, 215nm, eluent heptane:isopropanol 99.5:0.5, elution time 10.7 min (*S*) and 11.6 min (*R*).

^{1}H NMR MIP-protected (*R*)-mandelonitrile (**5a**)



5 Delville, M. M. E.; van Gool, J. J. F; van Wijk, I. M.; van Hest, J. C. M.; Rutjes, F. P. J. T. *J. Flow Chem.* **2012**, *4*, 124-128.