

Utilising ^{14}C -Radiolabelled Atom Transfer Radical Polymerisation Initiators

Mark Long, David W Thornthwaite, Suzanne H Rogers, Gwénaëlle Bonzi, Francis R Livens
and Steve P Rannard

Supporting Information

Reagents and Suppliers

All reagents and solvents were purchased from the Sigma Aldrich Group unless otherwise stated. Prosafe liquid Scintillation Cocktail was purchased from Meridian Biotechnologies; PMMA GPC Standards were purchased from Polymer Laboratories; Partisil LK6DF Silica Gel 60A TLC Plates were purchased from BDH; ^{14}C Methyl Iodide (98.4%) was purchased from Amersham Biosciences; $^{14}\text{CH}_2$ Benzyl Alcohol (95%) was kindly supplied by Unilever.

Instrumentation Analysis and Sample Preparation

NMR

All ^1H NMR and ^{13}C NMR spectroscopy was conducted using a Bruker Advance DRX500 spectrometer. Sample preparation for ^1H NMR involved approximately 2mg of the sample dissolved in 1ml of CDCl_3 , ^{13}C NMR involved approximately 50mg of the sample dissolved in 1ml of CDCl_3

Liquid Scintillation Counter

The analysis of all radioactive samples was conducted using a Packard Tri Carb 3100 TRC Liquid Scintillation Counter. The results are presented as Disintegrations Per Minute (DPM)

All Liquid Scintillation samples comprised a 20ml scintillation vial containing a known mass of radioactive compound dissolved in an appropriate solvent and 10ml of Prosafe Liquid Scintillation Cocktail containing:

60-75% Phenyl Xylyl Ethane
20-40% Alcohol Ethoxylate
2-8% Alcohol Ether Phosphate Ester
0.1-1.0% 2,5-Diphenyloxazole
0.1-1.0% 1,4-Bis(4-methyl-alpha-styryl)benzene

Gel Permeation Chromatography (GPC)

The GPC analyses were conducted in THF using instrumentation comprising:

Agilent 11100 Series RID with a response time of < 0.2min

40 $^\circ\text{C}$ Eppendorf Oven

Jasco PU 1580 Pump

Jasco AS 590 Auto Samplers

Polymer Laboratories PLgel 5 μm mixed-C and PLgel 5 μm mixed-D columns with a PL guard column

The eluent flow rate was 0.8 ml/min.

Calibration was conducted using poly methyl methacrylate standards with molecular weights ranging between 1000 and 1.5 million.

Radio TLC (R-TLC)

R-TLC analysis was conducted using an AR 2000 BIOSCAN Radio TLC Imaging Scanner utilising a gas filled proportional counter filled with 90/10 Argon/Methane to detect the beta radioactive emissions from thin layer chromatography (TLC) plates.

The TLC samples were (1.0% w/w) prepared in an appropriate solvent and eluted using Whatman TLC plates (Partisil LK6DF silica Gel 60A with FL indicator) with a silica thickness of 250 μm and 3cm pre adsorbent zone. The plates were eluted using an appropriate solvent to a standard 15cm solvent front. After drying the plates were scanned using the AR 2000 BIOSCAN LSC.

Mass Spectrometry

Mass spectrometry was conducted using a Micromass LCT Mass Spectrometer using a cone voltage of 50 Volts. Samples were prepared in methanol at concentrations of approximately 1 mg ml $^{-1}$

Synthetic Procedures

A) Synthesis of Benzyl 2-bromo isobutyrate

To a 250ml one neck round bottomed flask containing a magnetic stirrer flushed with nitrogen was added dry dichloromethane (150ml), benzyl alcohol (0.1 mol), triethylamine (1.1eq) and dimethyl amino pyridine (0.013eq). The reaction mixture was cooled to 0°C using an ice water bath. With stirring, bromo isobutyryl bromide (1.1eq) was added dropwise to the reaction mixture using a pressure equalizing dropping funnel. Once this addition was complete, the reaction flask was left to stir for twenty four hours initially at 0°C but allowed to warm to room temperature.

The reaction mixture was evaporated to remove the solvent to yield a crude yellow oil and a cream precipitate. Dilute hydrochloric acid and diethyl ether were added and the product was washed 4 times. Finally the mixture was washed with dilute sodium carbonate. The combined diethyl ether layers were dried over sodium sulphate, filtered and evaporated to yield a bronze coloured oil product. The structure and purity of the final product was confirmed by ¹H NMR, ¹³C NMR and Mass Spectrometry. Chemical purity > 95%. Chemical Yield 23.9g (93%).

Figure S1: ¹H NMR spectrum of Benzyl 2-bromo isobutyrate

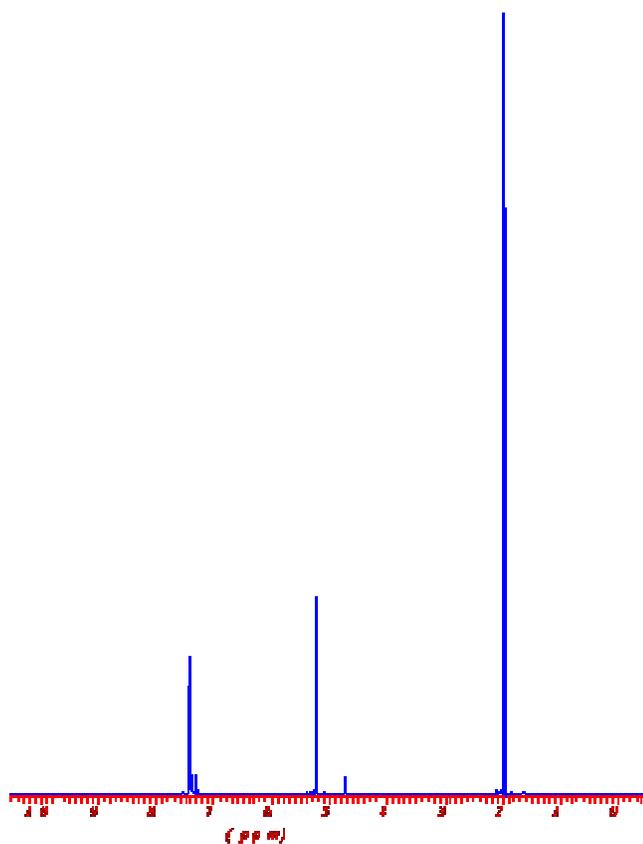


Figure S2: ^{13}C NMR spectrum of Benzyl 2-bromo isobutyrate

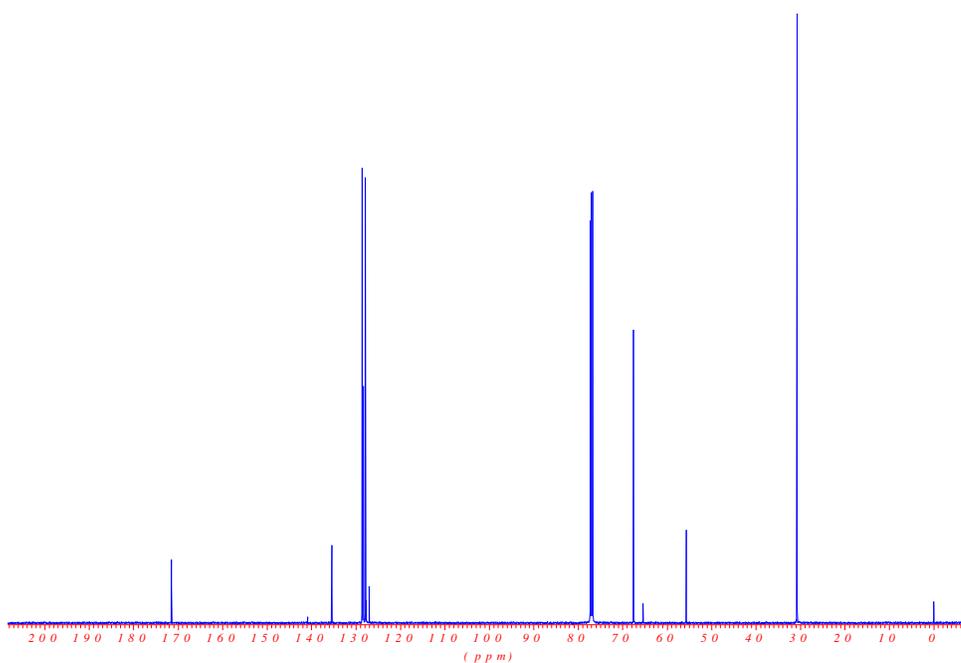
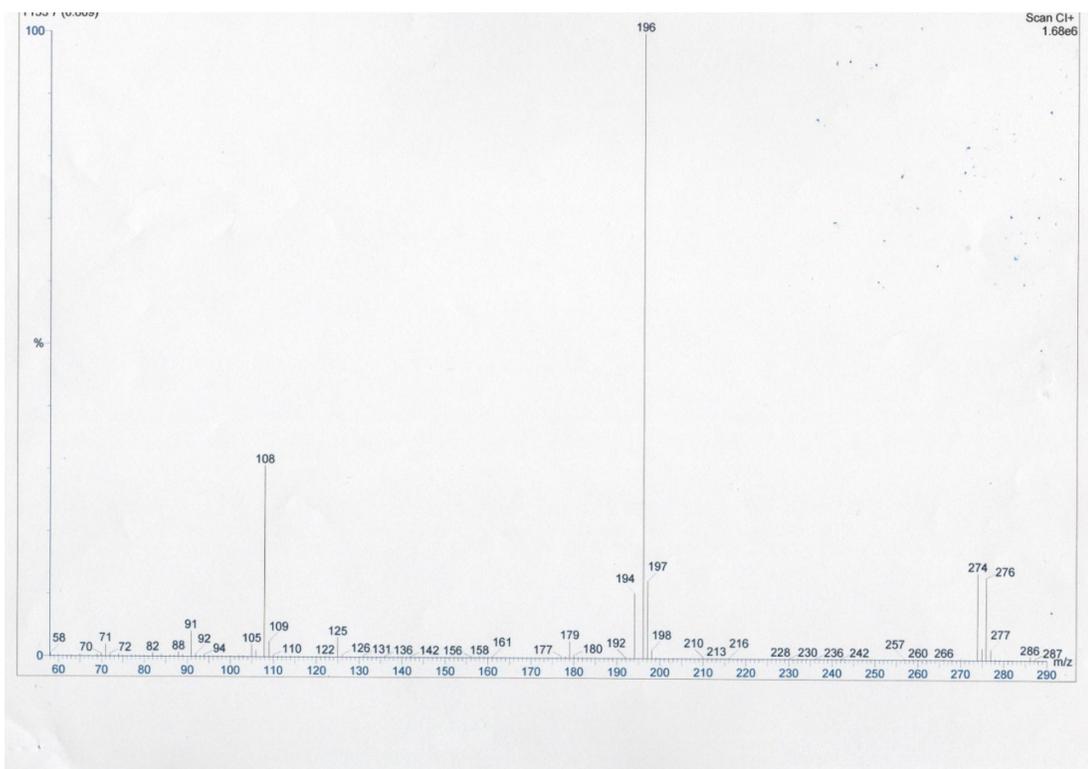


Figure S3: Mass Spectrum of Benzyl 2-bromo isobutyrate



Theoretical Mass = 274 Daltons

B) Synthesis of 3

The synthesis of 3 utilised benzyl ($^{14}\text{CH}_2$) alcohol (0.0203 moles, 13.56mCi) and followed the procedure described above. The radiochemical purity was determined by R-TLC using 3 eluents 90/10, 40-60 Petroleum ether/ diethyl ether, 95/5 40-60 Petroleum ether/diethyl ether and 100% dichloromethane. Total activity and specific activity were determined. Chemical Yield 4.5g (87%), Radiochemical Yield 97% Total Activity 13.13 mCi Specific Activity 3.015 uCi/mg Chemical Purity > 95% Radiochemical Purity 96%

Figure S4: ^1H NMR spectrum of 3

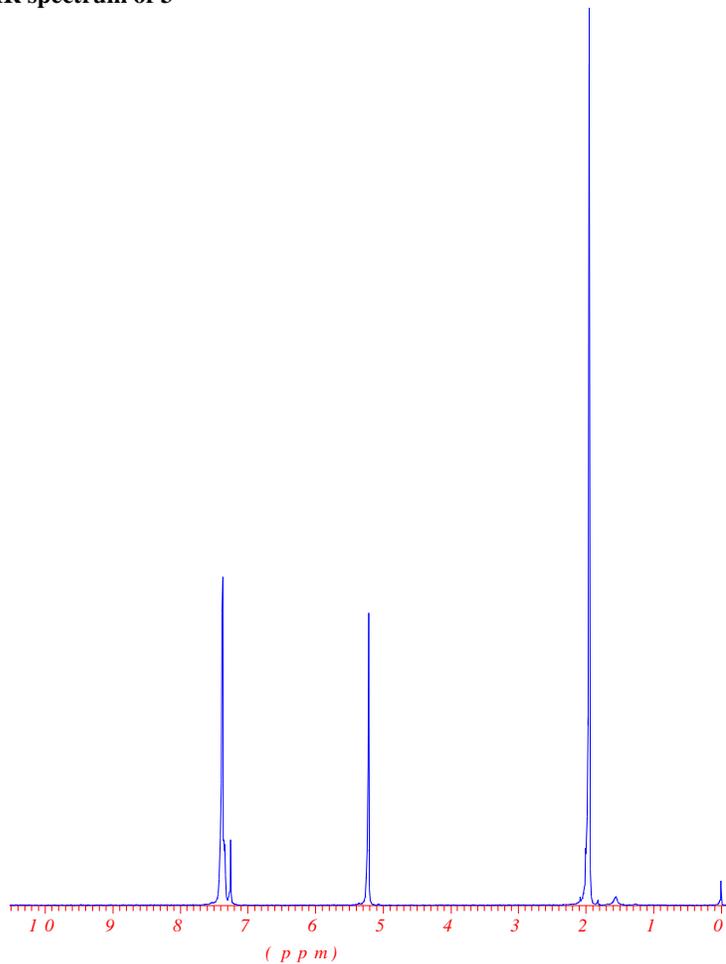


Figure S5: ^{13}C NMR spectrum of **3**

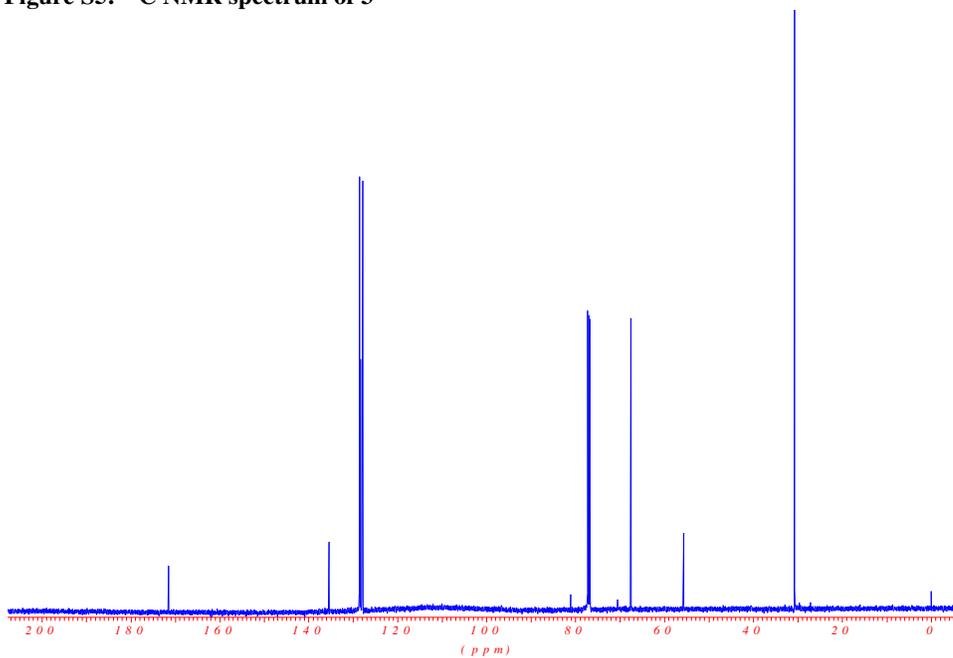
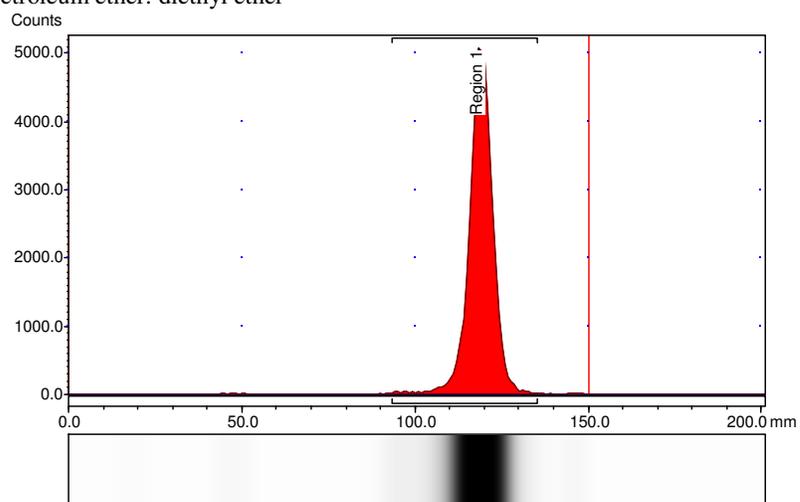
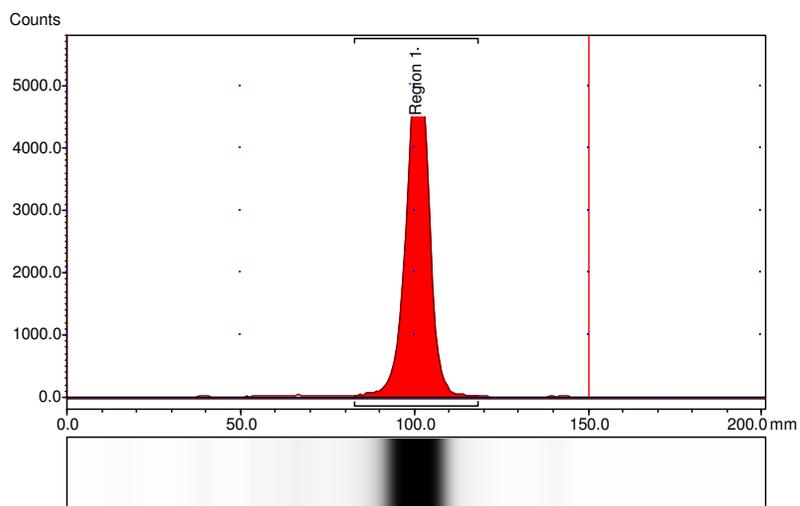


Figure S6: R-TLC of 3 in various solvent mixtures

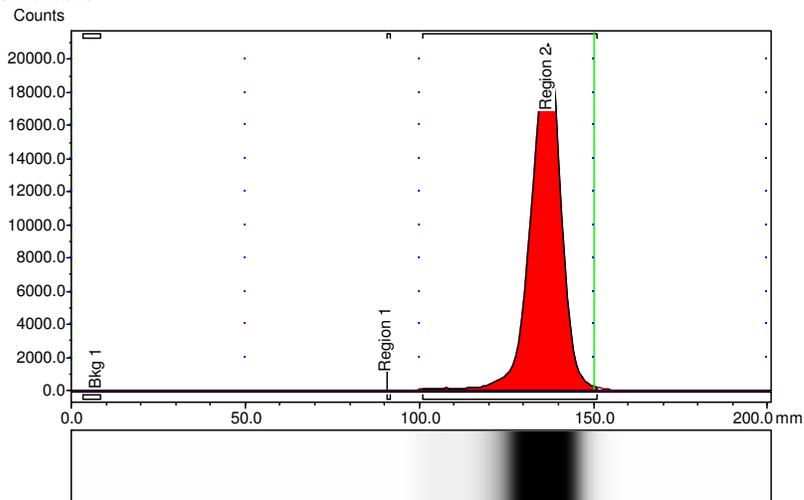
90/10, 40-60 Petroleum ether: diethyl ether



95/5, 40-60 Petroleum ether: diethyl ether



100% Dichloromethane

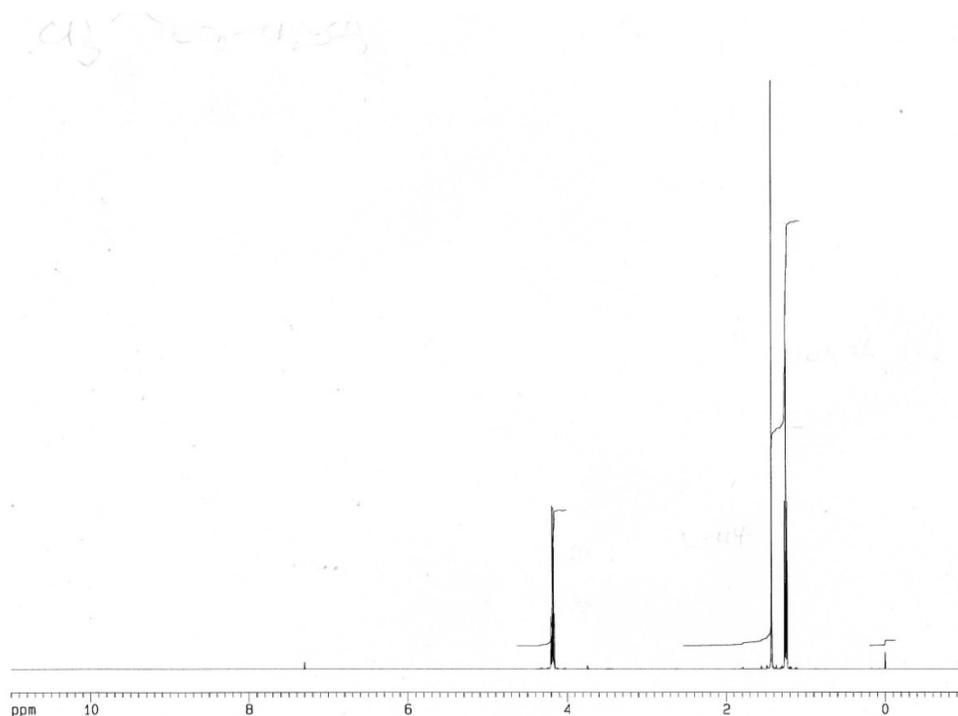


C) Synthesis of Non Labeled 2-bromo isobutyric acid

Step 1 Methylation of Diethyl Methyl Malonate

To a 50ml one necked round bottom flask containing a magnetic stirrer was added of diethyl methyl malonate (5.9 mmol), sodium ethoxide (21% solution, 1.1eq) and ethanol (10ml) under nitrogen. The reaction mixture was stirred, and warmed to 50°C for 30 minutes. The reaction mixture was then cooled to room temperature. Methyl iodide (8.38 mmol, 1.4 eq) was added. The flask was flushed with nitrogen, sealed and warmed to 50°C for 4 hours. After cooling, the ethanol and unreacted methyl iodide were removed using a rotary evaporator. Dry diethyl ether was added to the residue and the sodium iodide was filtered to yield a clear crude solution of diethyl dimethyl malonate. The diethyl ether was washed with water to remove residual sodium iodide. The diethyl ether layer was dried over magnesium sulphate, filtered and evaporated to yield a clear bronze coloured liquid. The structure and chemical purity of the final product was confirmed by ¹H NMR Chemical Yield 0.81g (73%), Chemical Purity > 95%

Figure S7: ¹H NMR spectrum of diethyl dimethyl malonate



Step 2 Hydrolysis of diethyl dimethyl malonate

To the diethyl dimethyl malonate, was added 2.4eq of an aqueous solution of Potassium Hydroxide (10ml). The solution was warmed to 80°C with stirring until the oil was completely dissolved.

Step 3 Decarboxylation of the di-Potassium salt of dimethyl malonate

To the aqueous solution of the di-Potassium salt of dimethyl malonate was added an aqueous solution of sulphuric acid (2.4 eq, 20ml). The mixture was refluxed for five hours. The resulting isobutyric acid was recovered via distillation (azeotroped with water). To the water/isobutyric acid mixture potassium hydroxide was added (1.2eq). The solution was then freeze dried to yield potassium isobutyrate

Step 4 Bromination of Potassium Isobutyrate

Dry dichloroethane (30ml) and hydrochloric acid (2 eq) were added to the 50ml round bottomed flask containing the potassium isobutyrate. The reaction mixture was stirred for one hour at ambient temperature and purged with nitrogen. Chlorosulphonic acid (0.75 eq) and bromine (1 eq) were added. The reaction mixture was refluxed under nitrogen for six hours, then evaporated to remove solvent, excess hydrochloric acid, and residual bromine. The oil was redissolved in diethyl ether and washed with water to remove residual bromine and free propanoic acid. The ether was dried over magnesium sulphate, filtered and evaporated. The structure and purity of the final product, 2-bromo isobutyric acid, was confirmed by ¹H NMR and ¹³C NMR. Overall Chemical Yield 550mg (56%).

Figure S8: ^1H NMR of 2-Bromo isobutyric acid

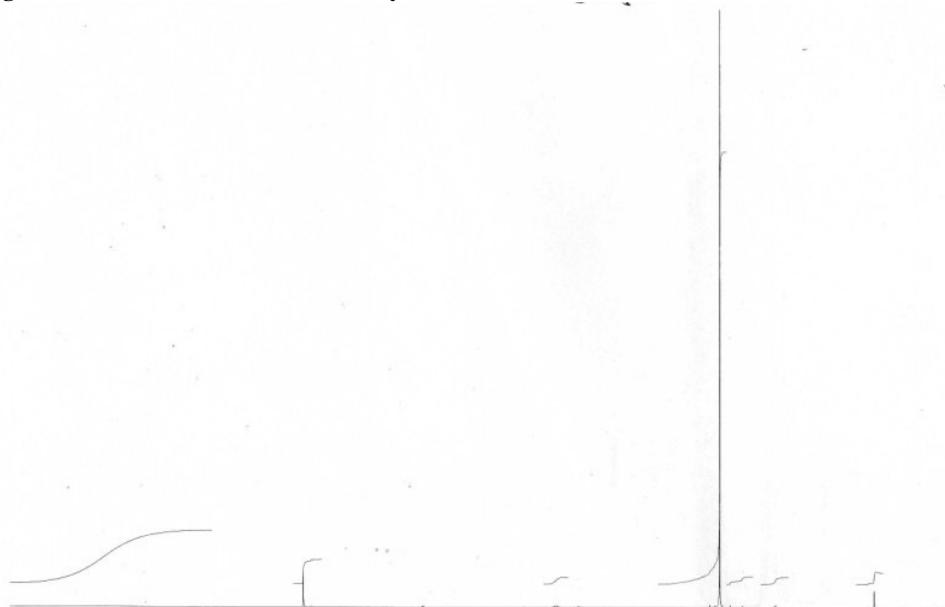
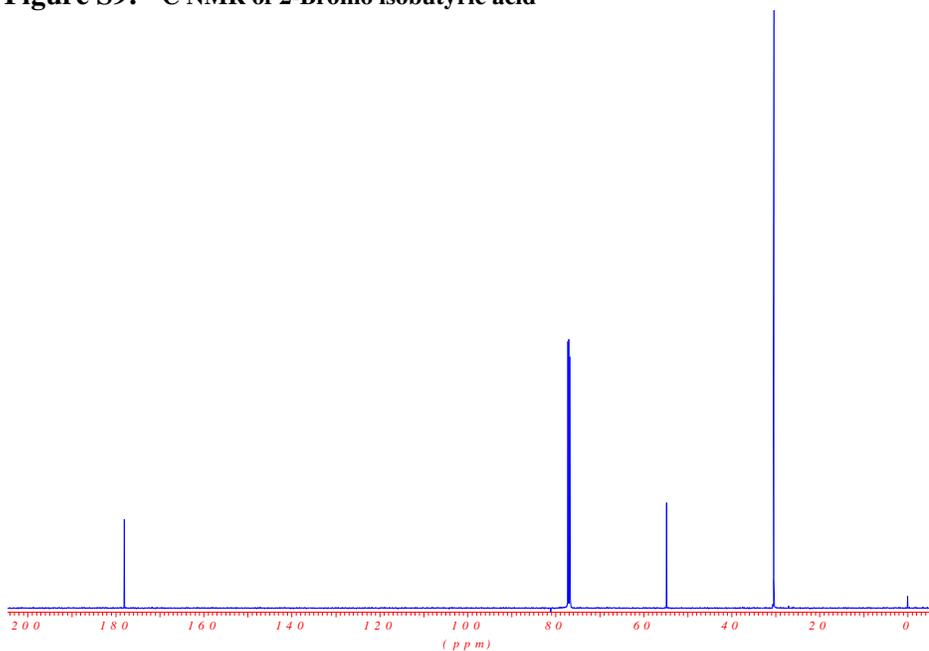


Figure S9: ^{13}C NMR of 2-Bromo isobutyric acid

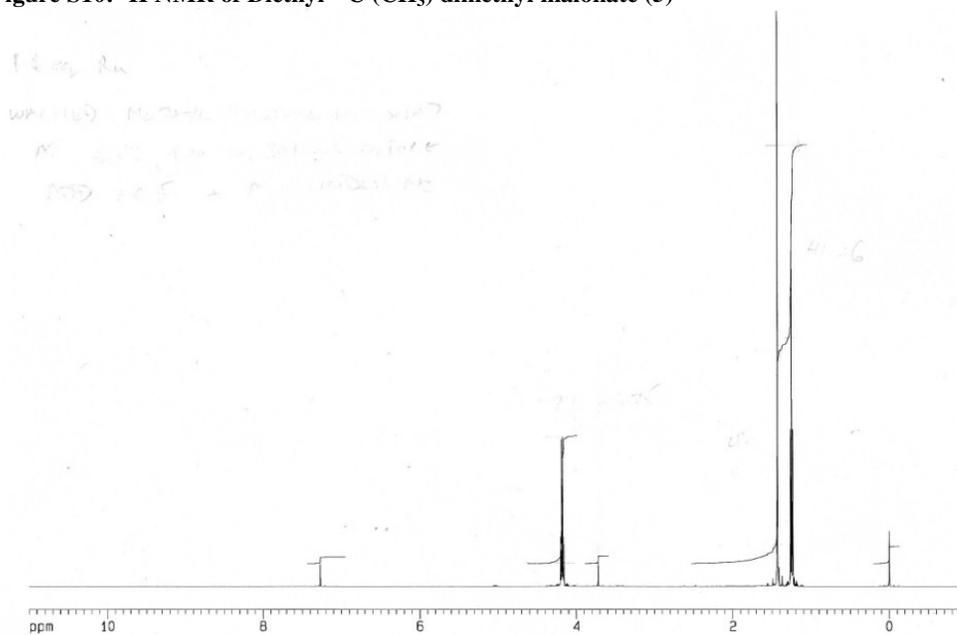


D) Synthesis of 2-Bromo ^{14}C (CH_3) isobutyric acid (11)

^{14}C (CH_3) Methylation of diethyl methyl malonate

To a 50ml one necked round bottom flask containing a magnetic stirrer was added of diethyl methyl malonate (5.9 mmol), sodium ethoxide (21% solution, 1.1eq) and ethanol (10ml) under nitrogen. The reaction mixture was stirred under a nitrogen atmosphere, and warmed to 50°C for 30 minutes. The reaction mixture was left to cool to room temperature. Methyl iodide was added in two aliquots (initial addition 10 mCi/0.18mmoles of ^{14}C -methyl iodide (8.38 mmol, 1.4 eq) from a sealed ampoule followed by non-labelled methyl iodide (8.2mmoles)). The flask was flushed with nitrogen and warmed to 50°C for 4 hours. Solvent and unreacted methyl iodide were removed via evaporation. Dry diethyl ether was added to the residue and the sodium iodide was filtered to yield a clear crude solution of diethyl ^{14}C (CH_3) dimethyl malonate. The diethyl ether was washed with water to remove any residual sodium iodide. The collected diethyl ether layers were dried over magnesium sulphate, filtered and evaporated to yield a clear bronze coloured liquid. Chemical Yield 76%, Radiochemical Yield 81% Total Activity 8.13 mCi Specific Activity 9.64 uCi/mg Chemical Purity > 95%

Figure S10: ^1H NMR of Diethyl ^{14}C (CH_3) dimethyl malonate (5)



Subsequent steps were repeated as above to produce 2-bromo ^{14}C (CH_3) isobutyric acid, as confirm by NMR and R-TLC. The total activity and specific activity were also determined. Chemical Yield 35%, Radiochemical Yield 54% Total Activity 5.4 mCi Specific Activity 15.6 uCi/mg Chemical Purity > 95% Radiochemical Purity 99%

Figure S11: ^1H NMR of 2-Bromo ^{14}C (CH_3) isobutyric acid (8)

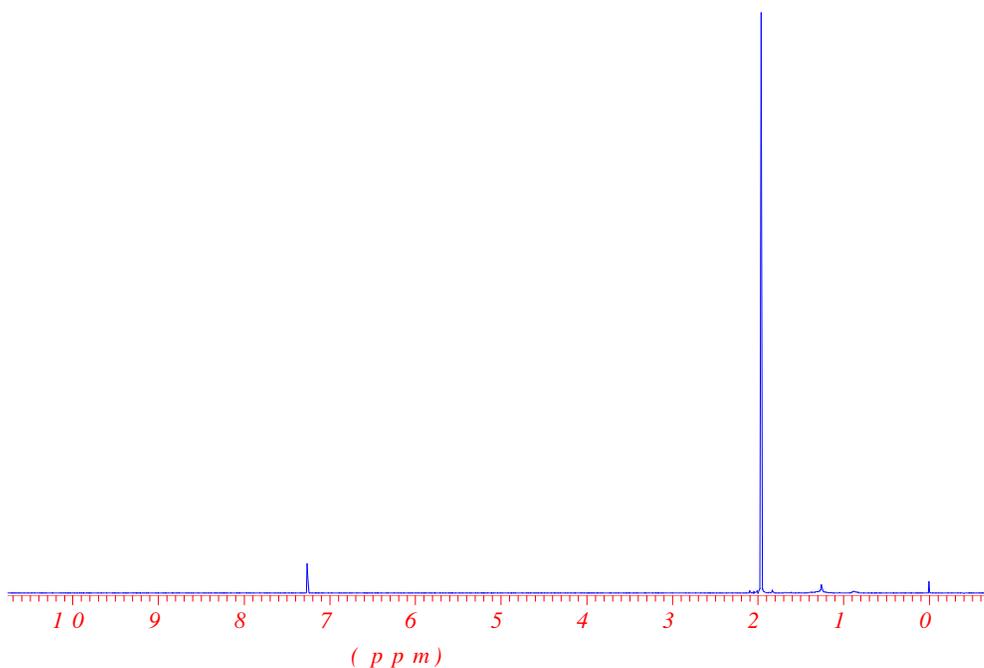


Figure S12: ^{13}C NMR of 2-Bromo ^{14}C (CH_3) isobutyric acid (**8**)

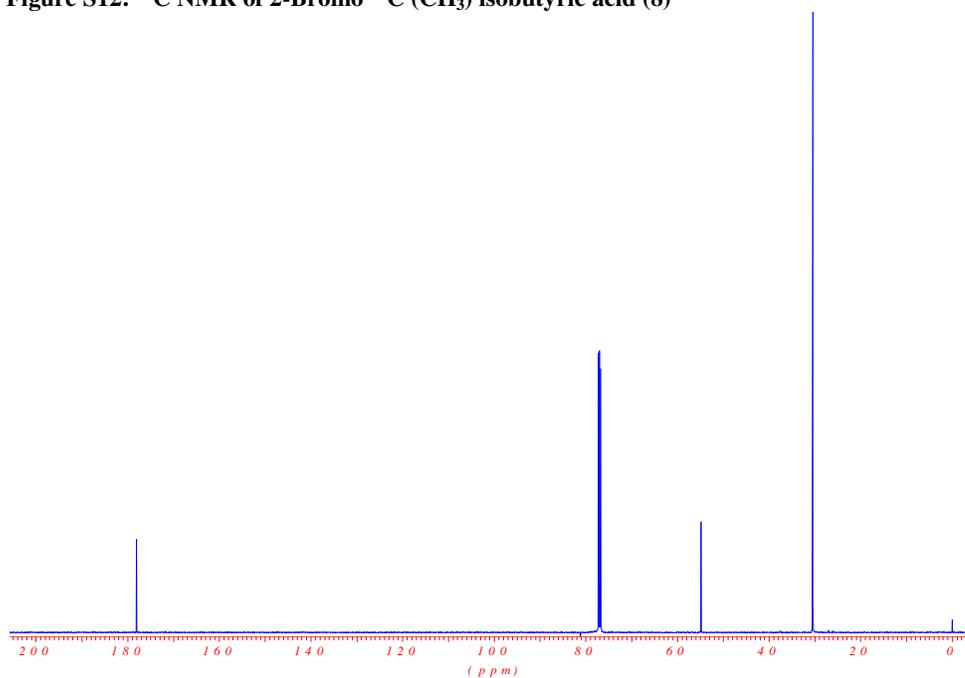
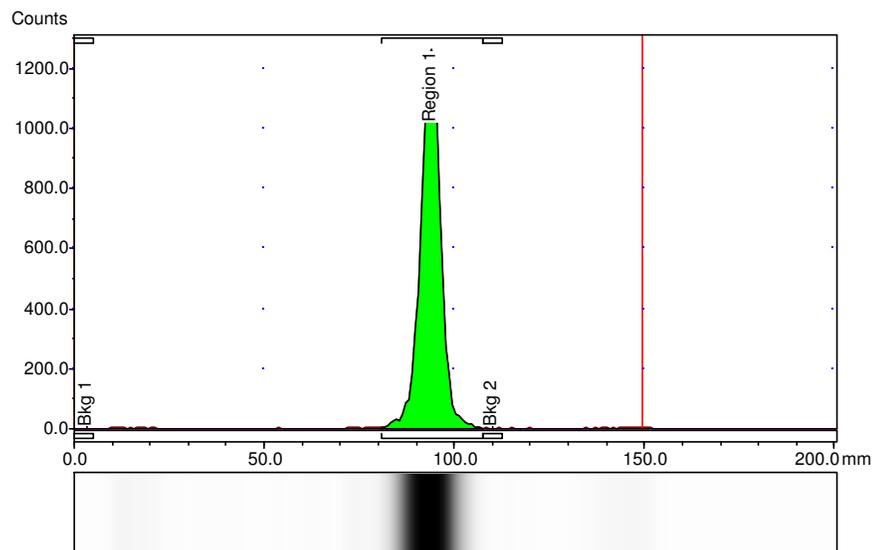


Figure S13: Radioanalytical TLC of Bromo ^{14}C (CH_3) isobutyric acid (**8**)



Synthesis of Benzyl 2-bromo ($^{14}\text{CH}_3$) isobutyrate (11)

To a 2 necked 50ml round bottomed flask was added 2-bromo isobutyric acid (2-bromo ($^{14}\text{CH}_3$) isobutyric acid (0.00051mol) and of non labelled 2-bromo isobutyric acid (0.00729mol) and dry Tetrahydrofuran (25ml). The reaction mixture was warmed under nitrogen to 60°C then 1,1'-carbonyldimidazole (1.0eq) was added. The mixture was stirred and heated under nitrogen at 60°C until the 1,1'-carbonyldimidazole was totally dissolved and the effervescence (liberation of CO_2) ceased. At this point, benzyl alcohol (1.0eq) was added and the reaction mixture left to reflux for 4 hours. The reaction mixture was evaporated and the residue was redissolved in diethyl ether. The crude product was first washed with dilute hydrochloric acid followed by washing with aqueous sodium carbonate. The water layers were further extracted with diethyl ether. All diethyl ether layers were combined and dried over sodium sulphate, filtered and evaporated to yield a water clear oil. The structure of the final product, Benzyl 2-bromo ($^{14}\text{CH}_3$) isobutyrate was confirmed by ^1H NMR and ^{13}C NMR and the chemical and radiochemical purities determined by ^1H NMR and R-TLC using 3 eluents 90/10, 40-60 Pet Ether: Ether, 95/5 40-60 Pet Ether; Ether and 100% Dichloromethane The total activity and specific activity determined. Chemical Yield 1.1g (55%), Radiochemical Yield 56%, Total Activity 0.397mCi, Specific Activity 0.362uCi/mg, Chemical Purity 97%, Radiochemical Purity 98%

Figure S14: ^1H NMR of Benzyl 2-bromo ($^{14}\text{CH}_3$) isobutyrate (11)

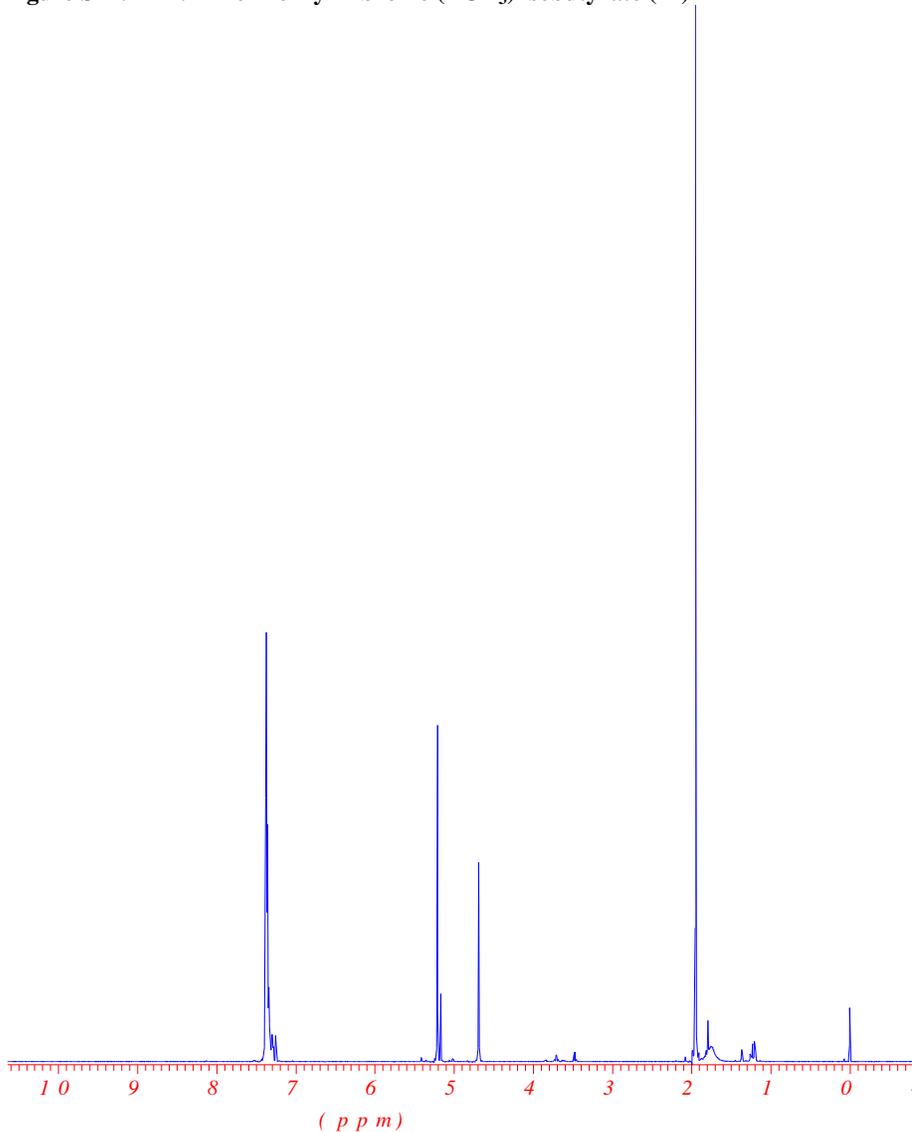


Figure S15: ^{13}C NMR of Benzyl 2-bromo ($^{14}\text{CH}_3$) isobutyrate (**11**)

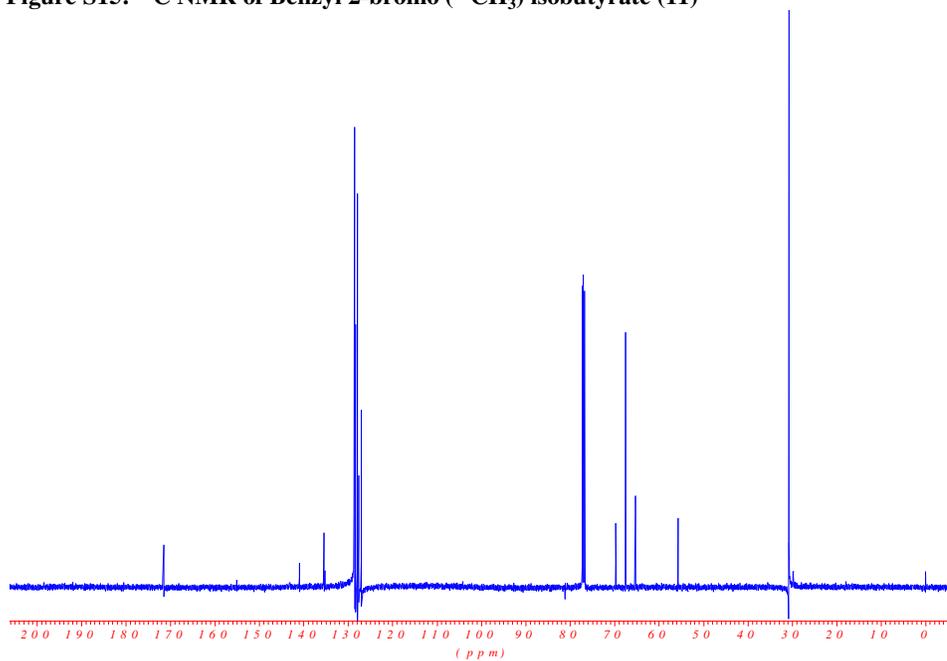
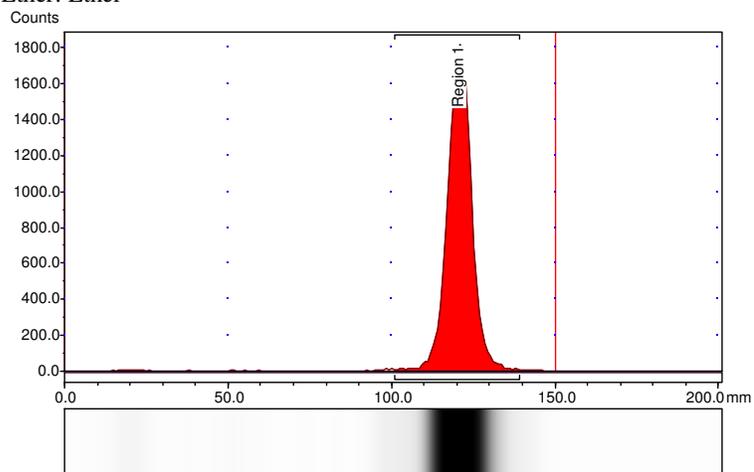
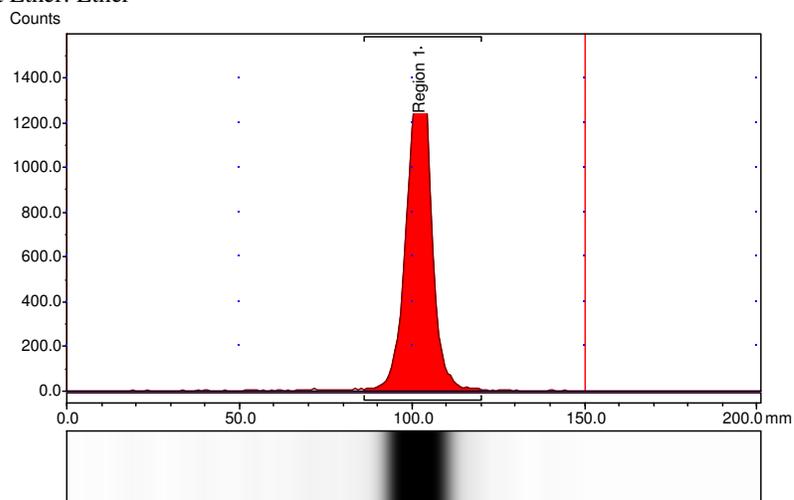


Figure S16: R-TLC Trace of Benzyl 2-bromo (^{14}C) isobutyrate (11)

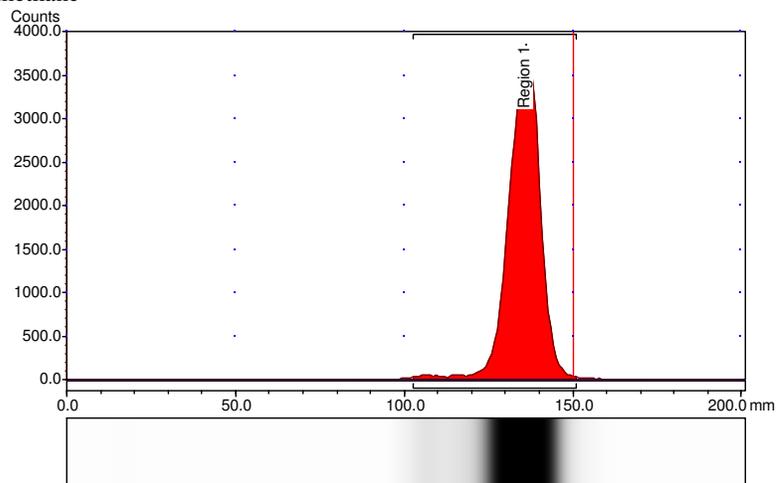
90/10, 40-60 Pet Ether: Ether



95/5, 40-60 Pet Ether: Ether



100% Dichloromethane



Rf values

Elution Solvent	Observed Rf value
100% 40/60 PET ETHER	0.276
95/5 40/60 PET ETHER/DIETHYL ETHER	0.615
100% DICHLOROMETHANE	0.914

Figure S17: Kinetic analysis of ATRP polymerisation of 2-HPMA using radiolabelled and non-labelled initiators.

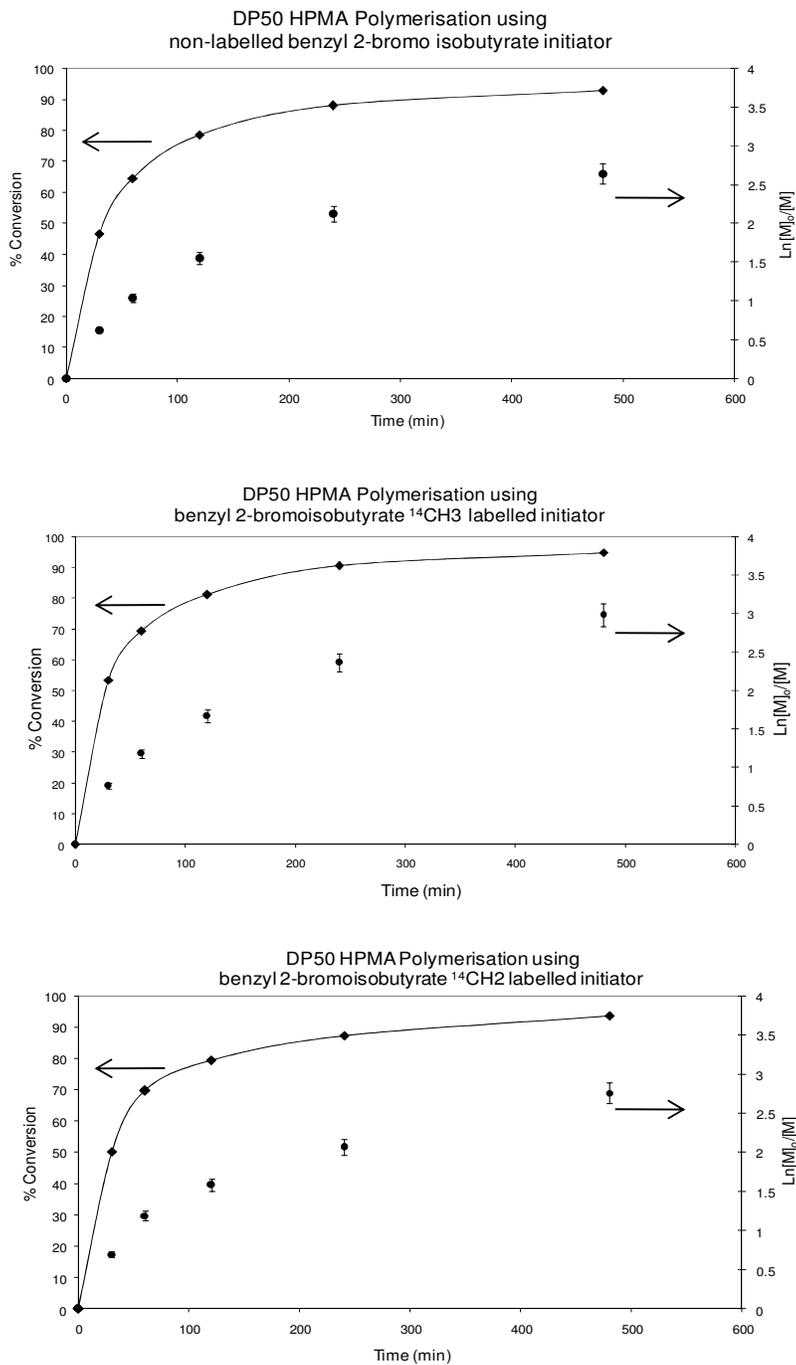
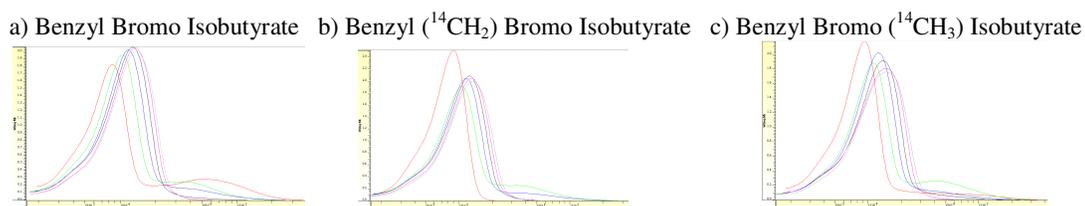


Figure S18: Overlaid GPC chromatograms of samples taken during the ATRP polymerisations of 2-HPMA.



NMR & GPC data of 2-HPMA ATRP polymerisation with a target $\text{DP}_n = 50$ monomer units.

	Initiator type	^1H NMR (calc)		Observed GPC			
		DP_n	M_n	DP_n	M_n	M_n	PDI
Poly(2-HPMA) (Target $\text{DP}=50$)	Non-labelled	40	5767	63	9014	12269	1.36
Poly(2-HPMA) (Target $\text{DP}=50$)	$^{14}\text{CH}_2$ - labelled	38	5479	62	8913	11999	1.35
Poly(2-HPMA) (Target $\text{DP}=50$)	$^{14}\text{CH}_3$ - labelled	37	5334	67	9640	14091	1.46

Figure S19: R-TLC of Poly (2-Hydroxy propyl methacrylate) using THF as eluent

