Electronic Supplementary Material (ESI) for Digital Discovery.

Digital Design of 3D Printed Plug-&-Play Reactionware for on Demand Synthesis of High Value Probes

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Contents

1 General Experimental Remarks

3D printing was achieved on Ultimaker 2+ FDM 3D printers supplied by Ultimaker and modified by the authors to print with polypropylene. ^{1}H, $^{13}C\{^{1}H\}$ spectra were recorded on a Bruker Avance III HD 600 MHz and Bruker Avance II 400 MHz spectrometers. Chemical shifts are reported in ppm relative to residual solvent or 3-(Trimethylsilyl)propionic acid-d4 sodium salt where appropriate. Multiplicities are given as s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, with coupling constants reported in Hz. Purities of the products are were determined by HPLC.

Mass spectrometry was carried out on a Bruker MaXis Impact quadrupole time-of-flight mass spectrometer with an electrospray source. The mass spectrometer was regularly calibrated using an Agilent ESI-L Low Concentration Tuning Mix 100m solution. Samples were introduced into the MS at a dry gas temperature of 200 °C and a dry gas flow rate of 10 mL/min. The ion polarity for all MS scans recorded was negative, with the voltage of the capillary tip set at 4500 V, end plate offset at − 500 V, funnel 1 RF at 400 Vpp, funnel 2 RF at 400 Vpp, ISCID at 0 eV hexapole RF at 100 Vpp, ion energy 5.0 eV, collision energy at 5 eV, collision cell RF at 200 Vpp, transfer time at 63.5 µs, and the pre-pulse storage time at 1.0 µs. The mass range was set to 50 – 2000 m/z. Data was analysed using the Bruker DataAnalysis v4.1 software suite. Additional mass spectrometry of NHS-diazirines were carried out using Jeol JMS-700 MStation equipped with FAB ion source.

The LC was carried out on a Thermo Dionex 3000 Ultimate HPLC system comprising of an LPG-3400RS pump, WPS-3000 autosampler, TCC-3000 column compartment and a DAD-3000 detector.

NHS-diazirine method:

Column:	Phenomenex LUNA 3µm HILIC 200Å (150 mm x 4.6mm)
Flow rate:	0.5 mL/min.
Column oven temp:	30°C
UV wavelengths:	190, 280, 300, 350 nm.

Method: isocratic 80% A, 20% B.

Sulfo-NHS-diazirine method :

Column: Phenomenex LUNA 3µm HILIC 200Å (150 mm x 4.6mm)

Flow rate: 0.4 mL/min.

Column oven temp: 20°C

UV wavelengths: 190, 214, 220, 254 nm.

Method:

Time (min)	A(%)	B(%)
0	3	97
4	10	90
16	70	30
19	100	0
23	3	97
26	3	97

2 Materials and Instruments

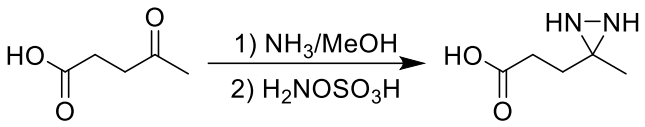
Solvents and reagents were used as received from commercial suppliers unless otherwise stated. Polypropylene feedstock for 3D printing was purchased from Barnes Plastic Welding Equipment Ltd., Blackburn, UK.

3 Traditional (Glassware) Synthesis of Target Materials

3.1 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate

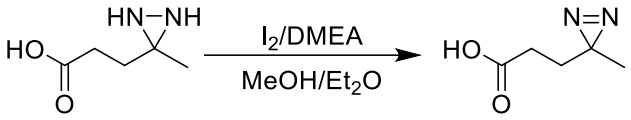
Certain elements of the procedure for the glassware synthesis of 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate were adapted from similar procedures found in literature.[1]

3.1.1 3-(3-methyldiaziridin-3-yl)propanoic acid



Precautions should be taken to avoid exposure to light throughout the synthesis. Levulinic acid (0.6 g, 5.167 mmol) was dissolved in anhydrous MeOH (1.5 mL) and transferred into 100 mL round bottom flask, flushed with nitrogen gas. The reaction vessel was closed with rubber septum and placed in a cooling bath at 0℃. 7M solution of ammonia in methanol (9 ml, 63 mmol, 12.2 equiv) was added into the reaction vessel. The mixture was stirred under nitrogen atmosphere for 1 h. The vessel was opened briefly to add 1.1 g of dried magnesium sulfate. The mixture was stirred for additional 5 h at 0℃. Hydroxylamine-O-sulfonic acid (0.701 g, 6.204 mmol, 1.2 equiv) was dissolved in 5.5 mL of anhydrous MeOH and added into the reaction vessel dropwise via syringe, over a course of 5 min. The reaction was then stirred for additional 16 h at 0℃. Subsequently the suspension was filtered off under flow of nitrogen gas and the resultant filtrate concentrated *in vacuo* (at 20℃ and 50 mbar), to afford oily off-yellow residue of the crude which was used immediately in the next step without purification.

3.1.2 3-(3-methyl-3H-diazirin-3-yl)propanoic acid



Crude 3-(3-methyldiaziridin-3-yl)propanoic acid was dissolved in anhydrous MeOH (7 mL) and stirred on an ice bath for 5 min, under argon. N,N-dimethylethylamine (0.57 mL, 5.26 mmol, 1.02 equiv) was added into the solution and this was stirred for further 5 min. Beads of iodine were then added gradually into the reaction mixture. When adding the iodine, colour of the reaction mixture became brown initially, but within a few seconds the colour disappeared to give a colourless solution again. Upon further addition of iodine, the rate of the disappearance of the brown colour slowed significantly. When the brown colour faded within 10 min of the last addition, giving clear yellow tinted solution at the end of the 10 min period, the addition of iodine was stopped. Note that 0.811 g, 3.195 mmol, 0.62 equiv of Iodine was normally required. The reaction was then left to stir for another 30 min. 3 mL of 50% w/v of KI solution was then added into the reaction, followed by 1.2 mL of 50% w/v solution of ascorbic acid and finally 1 mL of 3M HCl, added dropwise. The mixture was extracted immediately with diethyl ether. 30 mL of diethyl ether was used for first extraction, followed by two additional extractions, each using 20 mL aliquots of the solvent. The organic phase was dried over dried magnesium sulfate and the solvent evaporated in vacuo to afford the product as clear yellow, slightly viscous liquid. Note that care must be taken during evaporation of the solvent as high vacuum applied for extended periods of time will cause loss of yield due to evaporation of the product itself. For this reason, evaporation was generally carried out at 20-25°C and vacuum not exceeding 50 mbar. Following evaporation of the solvent the product may appear as a bi-phasal mixture which is caused by presence of KI as an impurity. We found that this had no apparent impact on the subsequent synthetic step and therefore used the compound without further purification. Estimated average yield 50%. ^{1}H NMR (600.1 MHz; 303 K; $CDCl_3$; δ, ppm; *J*, Hz): 10.89 (1H, bs), 2.22 (2H; t; 7.6), 1.70 (2H; t; 7.6), 1.03 (3H; s); $^{13}C\{^{1}H\}$ NMR (150.9 MHz, 303 K, $CDCl_3$; δ, ppm): 178.9, 29.5, 28.7, 25.2, 19.8.

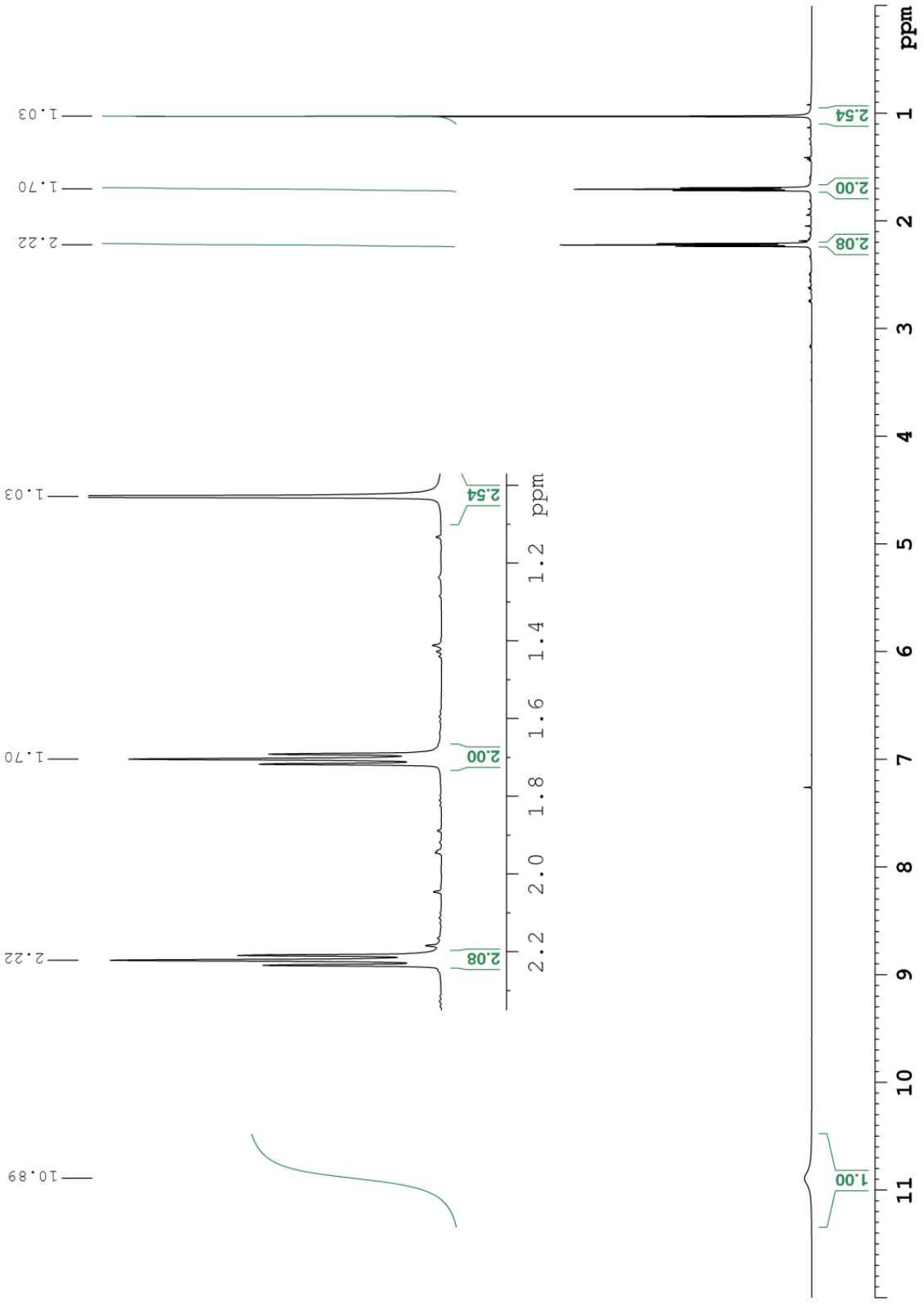


Figure 1: ^{1}H NMR of 3-(3-methyl-3H-diazirin-3-yl)propanoic acid.

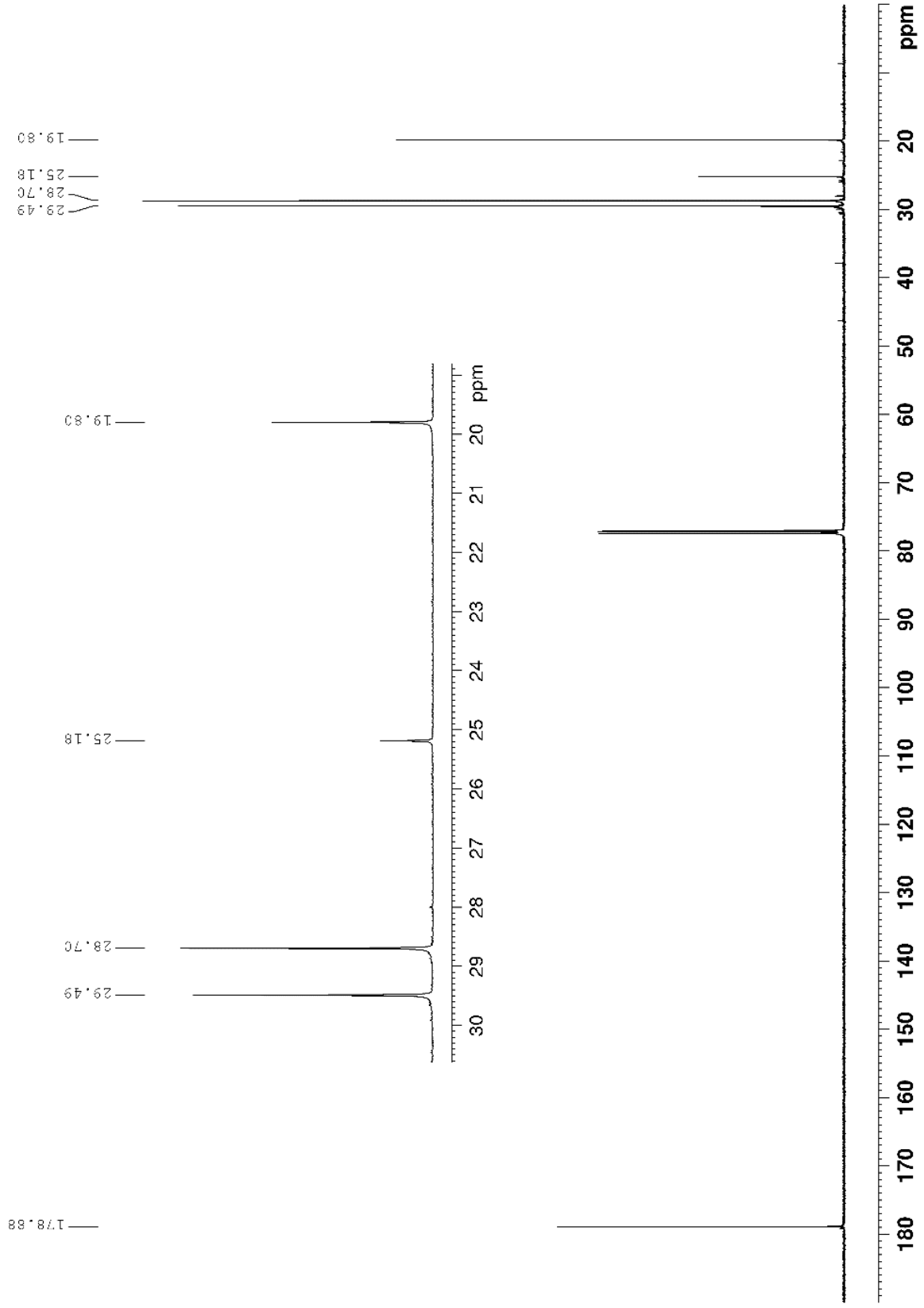
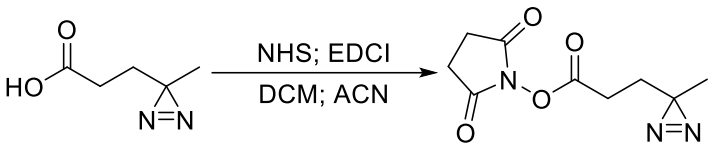


Figure 2: $^{13}C\{^{1}H\}$ NMR of 3-(3-methyl-3H-diazirin-3-yl)propanoic acid.

3.1.3 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate



The crude 3-(3-methyl-3H-diazirin-3-yl)propanoic acid (0.331 g, 2.583 mmol) was dissolved in 1.5 mL of anhydrous acetonitrile and stirred on an ice bath under argon. *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide (0.561 g, 3.616 mmol, 1.4 equiv) was dissolved in 3 mL of anhydrous DCM and added into the reaction vessel. *N*-hydroxysuccinimide (0.416 g, 3.616 mmol, 1.4 equiv) was dissolved in 3 mL of anhydrous acetonitrile and also added into the reaction. Finally, a few grains of freshly dried 3Å molecular sieves were also added and the vessel sealed with a rubber septum. The reaction mixture was stirred on an ice bath for 15 min. The ice bath was then replaced with a water bath set to 25℃ and the reaction stirred for another 18 h. The crude was then concentrated by evaporating the solvent partially at 25℃ and max 100 mbar vacuum, leaving roughly 2 mL of brown liquid in the vessel. This was purified by silica column chromatography using a mixture of anhydrous PET 40-60 and anhydrous ethyl acetate in 70:30 ratio, respectively. To aid transfer of the crude from the reaction vessel into the chromatography column, a small amount of anhydrous DCM (~1.5 mL) was used. Note that the silica was thoroughly dried* prior to use and loaded into column as a suspension in the eluent. Upon evaporation of the eluent, the product appeared as white solid with a final mass of 0.5 g, 2.22 mmol, yield 43% and 97% purity. ^{1}H NMR 600.1 MHz; 303 K; $CDCl_3$; δ, ppm; *J*, Hz): 2.83 (4H, bs), 2.51 (2H; t; 7.8), 1.80 (2H; t; 7.8), 1.07 (3H; s); $^{13}C\{^{1}H\}$ NMR (150.9 MHz, 303 K, $CDCl_3$; δ, ppm): 169.1, 167.8, 29.7, 25.9, 25.7, 24.9, 19.7.

*The silica was dried at 100-120℃ under vacuum (~10 mbar) for period of 3-4h. Subsequently it was allowed to return to room temperature and stored under argon.

3.1.4 NMR analysis of final product synthesized in glassware.

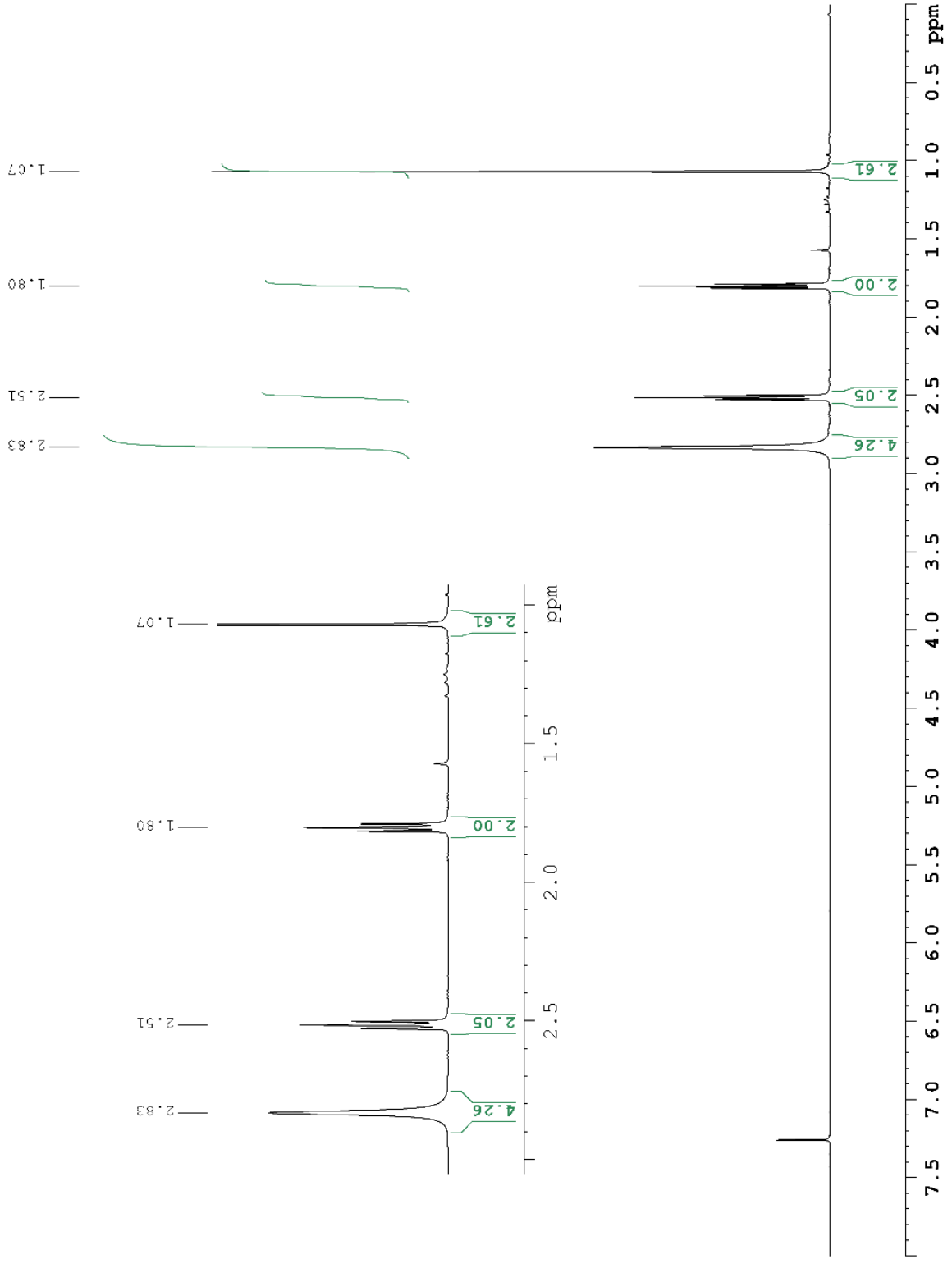


Figure 3: ^{1}H NMR of 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate.

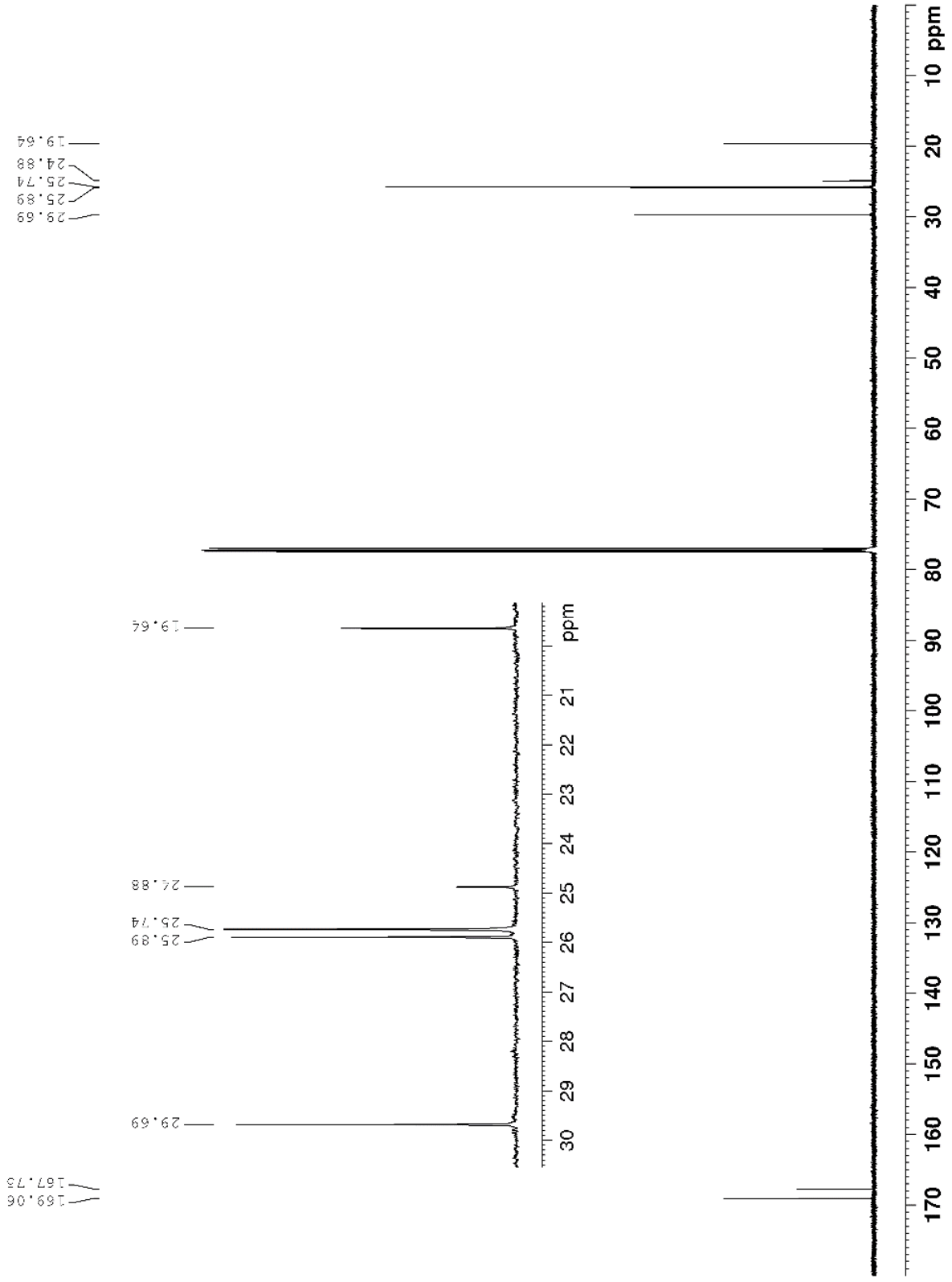
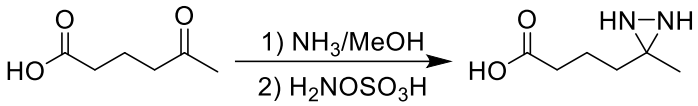


Figure 4: $^{13}C\{^{1}H\}$ NMR of 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate.

3.2 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate

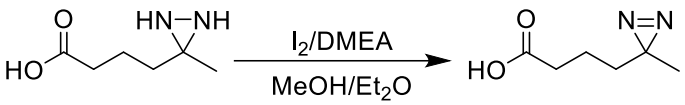
Certain elements of the procedure for the glassware synthesis of 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate were adapted from similar procedures found in literature.[1]

3.2.1 4-(3-methyldiaziridin-3-yl)butanoic acid



NOTE: Take precautions to avoid exposure to light throughout the synthesis. 4-Acetylbutyric acid (0.673 g, 5.17 mmol) was dissolved in anhydrous MeOH (2 mL) and transferred into 100 mL round bottom flask, flushed with nitrogen gas. The reaction vessel was closed with rubber septum and placed in a cooling bath at 0℃. 7M solution of ammonia in methanol (9 ml, 63 mmol, 12.2 equiv) was added into the reaction vessel. The mixture was stirred under nitrogen atmosphere for 1 h. The vessel was opened briefly to add 1.5 g of dried magnesium sulfate. The mixture was stirred for additional 5 h at 0℃. Hydroxylamine-O-sulfonic acid (0.701 g, 6.204 mmol, 1.2 equiv) was dissolved in 5.5 mL of anhydrous MeOH and added into the reaction vessel dropwise via syringe, over a course of 5 min. The reaction was then stirred for additional 16 h at 0℃. Subsequently the suspension was filtered off under flow of nitrogen gas and the resultant filtrate concentrated *in vacuo* (at 20℃ and 40 mbar), to afford off-white residue of the crude which was used immediately in the next step without purification.

3.2.2 4-(3-methyl-3H-diazirin-3-yl)butanoic acid



Crude 4-(3-methyldiaziridin-3-yl)butanoic acid was dissolved in anhydrous MeOH (7 mL) and stirred on an ice bath for 5 min, under argon. N,N-dimethylethylamine (0.57 mL, 5.26 mmol, 1.02 equiv) was added into the solution and this was stirred for further 5 min. Beads of iodine were then added gradually into the reaction mixture. When adding the iodine, colour of the reaction mixture became brown initially, but within a few seconds the colour disappeared to give a colourless solution again. Upon further addition of iodine, the rate of the disappearance of the brown colour slowed significantly. When the brown colour faded within 10 min of the last addition, giving yellow tinted solution at the end of the 10 min period, the addition of iodine was stopped. Note that 0.743 g, 2.927 mmol, 0.57 equiv of Iodine was normally required. The reaction was then left to stir for another 30 min. 3 mL of 50% w/v of KI solution was then added into the reaction, followed by 1.2 mL of 50% w/v solution of ascorbic acid and finally 1 mL of 3M HCl, added dropwise. The mixture was extracted immediately with diethyl ether. 30 mL of diethyl ether was used for first extraction, followed by two additional extractions, each using 20 mL aliquots of the solvent. The organic phase was dried over magnesium sulfate and the solvent evaporated in vacuo to afford the product as a clear yellow, slightly viscous liquid. Following evaporation of the solvent the product may appear as a bi-phasal mixture which is caused by presence of KI as an impurity. We found that this had no apparent impact on the subsequent synthetic step and therefore used the compound without further purification. Estimated average yield 40%. ^{1}H NMR (600.1 MHz; 303 K; $CDCl_3$; δ, ppm; *J*, Hz): 10.76 (1H, bs), 2.35 (2H; t; 7.3) 1.54 (2H; m), 1.41 (2H; t; 7.3), 1.02 (3H; s); $^{13}C\{^{1}H\}$ NMR (150.9 MHz, 303 K, $CDCl_3$; δ, ppm): 178.5, 33.8, 33.2, 25.6, 19.9, 19.4.

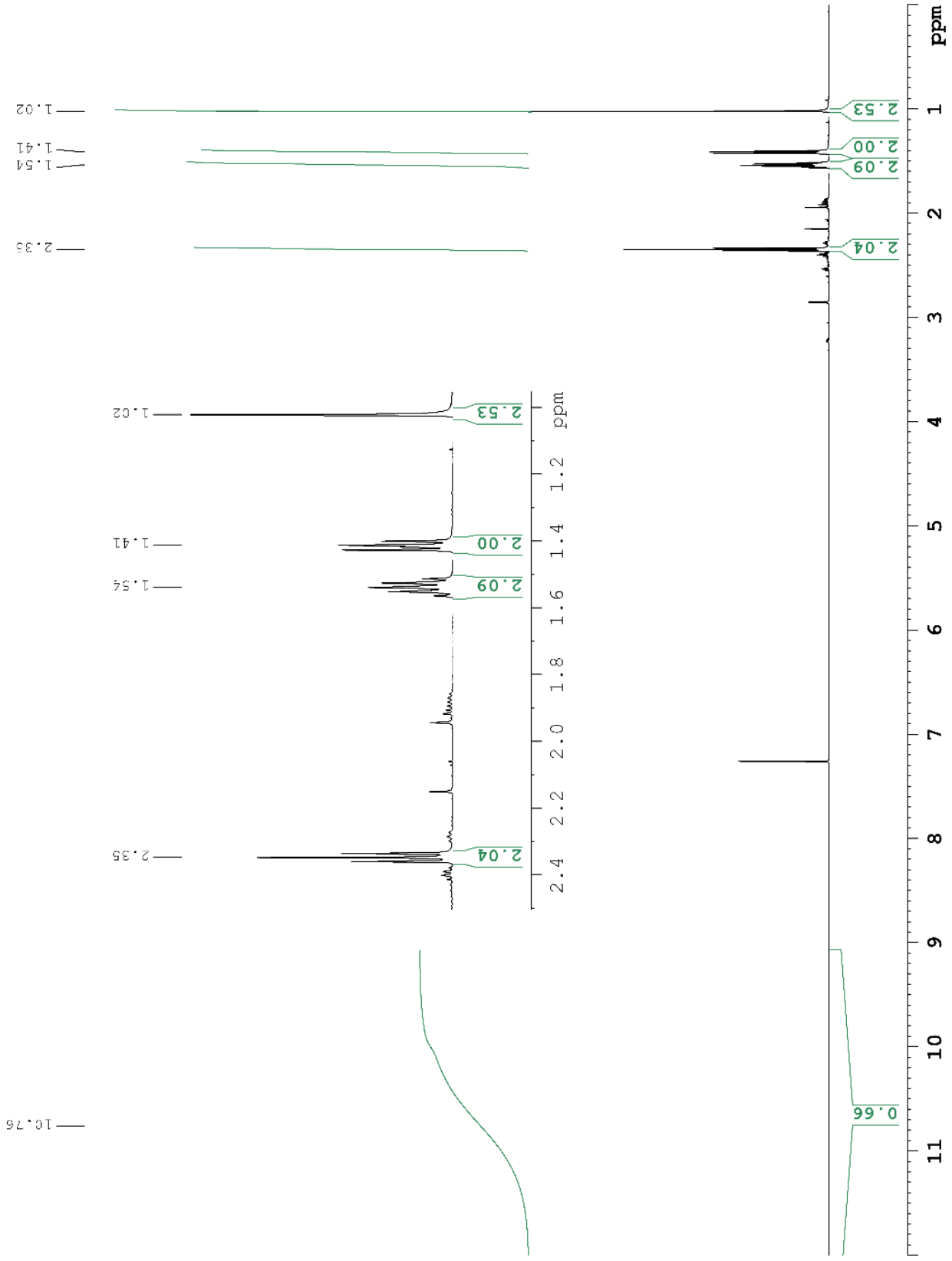


Figure 5: ^{1}H NMR of 4-(3-methyl-3H-diazirin-3-yl)butanoic acid.

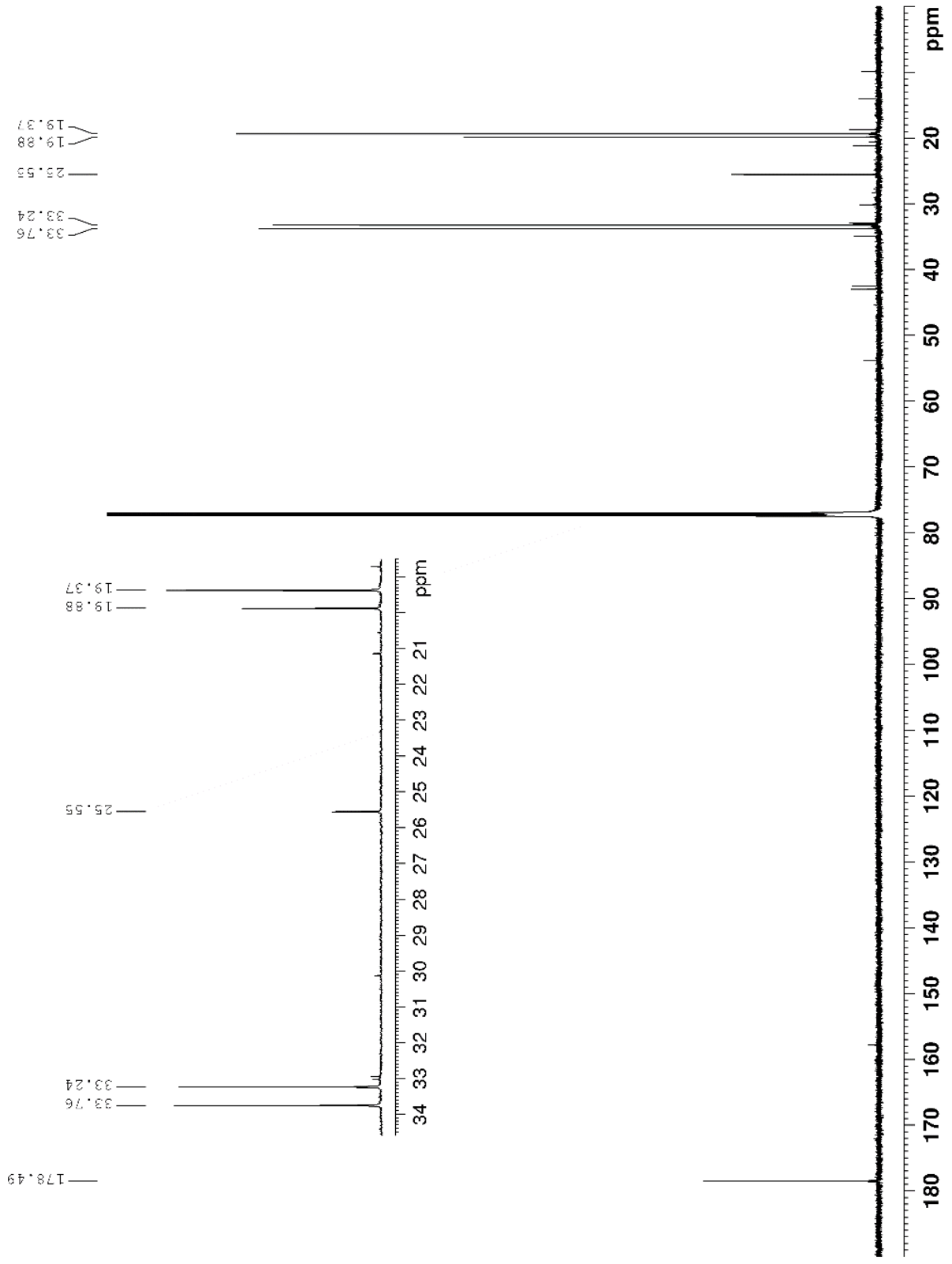
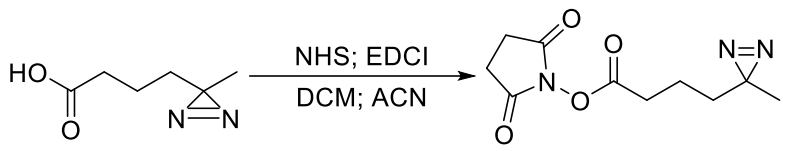


Figure 6: $^{13}C\{^{1}H\}$ NMR of 4-(3-methyl-3H-diazirin-3-yl)butanoic acid.

3.2.3 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate



The crude 4-(3-methyl-3H-diazirin-3-yl)butanoic acid (0.294 g, 2.068 mmol) was dissolved in 3 mL of anhydrous acetonitrile and stirred on an ice bath under argon. *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide (0.724 g, 4.666 mmol, 2.26 equiv) was dissolved in 4 mL of anhydrous DCM and added into the reaction vessel. *N*-hydroxysuccinimide (0.537 g, 4.666 mmol, 2.26 equiv) was dissolved in 4 mL of anhydrous acetonitrile and also added into the reaction. Finally, a few grains of freshly dried 3Å molecular sieves were also added and the vessel sealed with a rubber septum. The reaction mixture was stirred on an ice bath for 15 min. The ice bath was then replaced with a water bath set to 25℃ and the reaction stirred for another 18 h. The crude was concentrated by evaporating the solvent partially at 25℃ and max 100 mbar vacuum, leaving roughly 2.5 mL of brown liquid. This was purified by silica column chromatography using a mixture of anhydrous PET 40-60 and anhydrous ethyl acetate in 75:25 ratio, respectively. To aid transfer of the crude from the reaction vessel into the chromatography column, additional 2 mL of anhydrous DCM was used. Note that the silica was thoroughly dried* prior to use and loaded into column as a suspension in the eluent. Upon evaporation of the eluent, the product appeared as clear, off-yellow liquid. Note that after the initial evaporation of the eluent, the product contained traces of low boiling impurities originating from PET 40-60. To mitigate this the product was re-dissolved in 3 mL of deuterated chloroform and the solvent thoroughly evaporated again, giving material with a final mass of 0.367 g, 1.534 mmol, yield 30% and 99% purity. ^{1}H NMR (600.1 MHz; 303 K; $CDCl_3$; δ, ppm; *J*, Hz): 2.83 (4H, bs), 2.60 (2H; t; 7.3), 1.64 (2H; m), 1.48 (2H; t; 7.9), 1.03 (3H; s); $^{13}C\{^{1}H\}$ NMR (150.9 MHz, 303 K, $CDCl_3$; δ, ppm): 169.2, 168.2, 33.4, 30.4, 25.7, 25.4, 19.8, 19.4.

*The silica was dried at 100-120℃ under vacuum (~10 mbar) for period of 3-4h. Subsequently it was allowed to return to room temperature and stored under argon.

3.2.4 NMR analysis of final product synthesized in glassware.

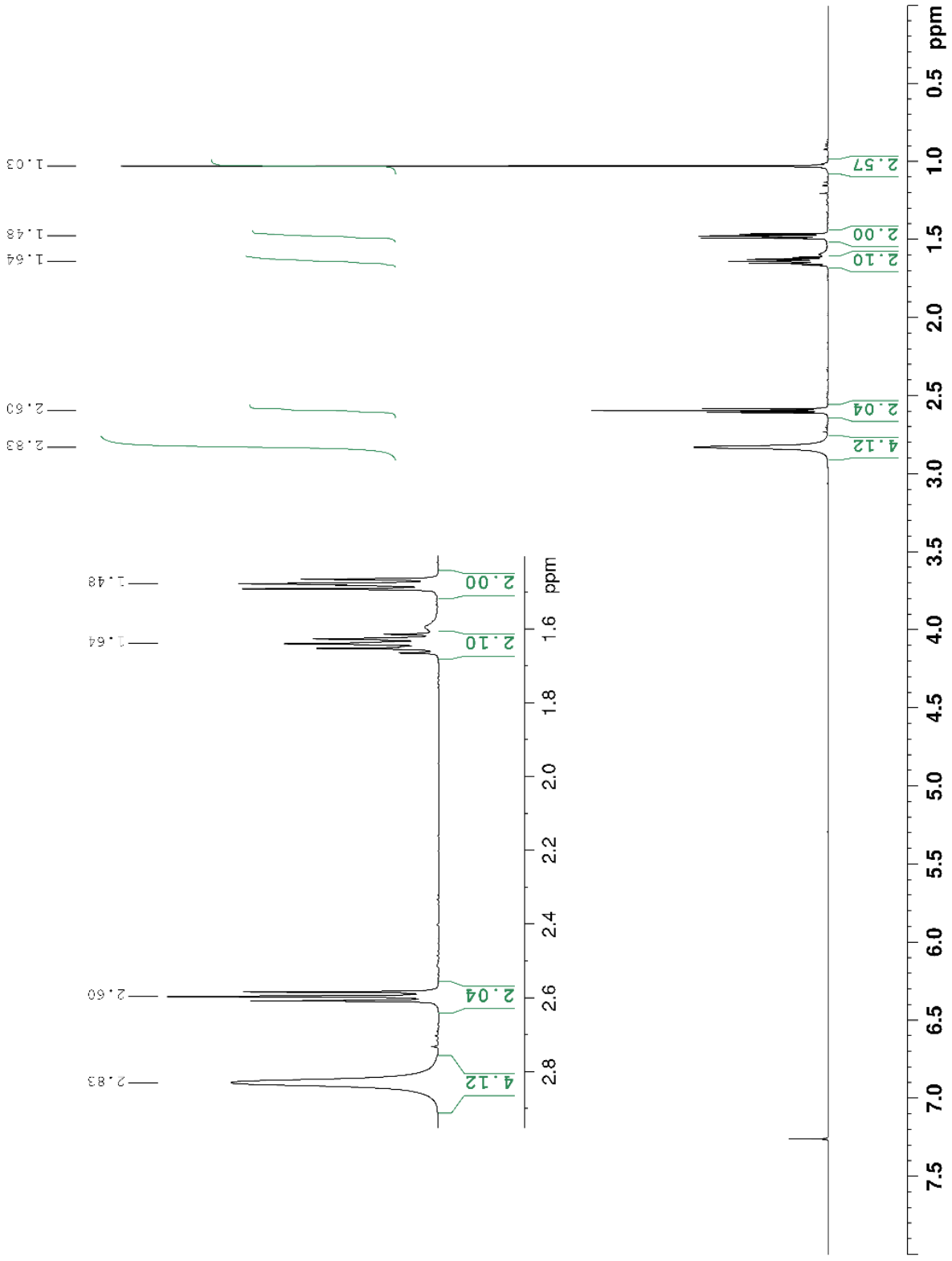


Figure 7: ^{1}H NMR of 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate.

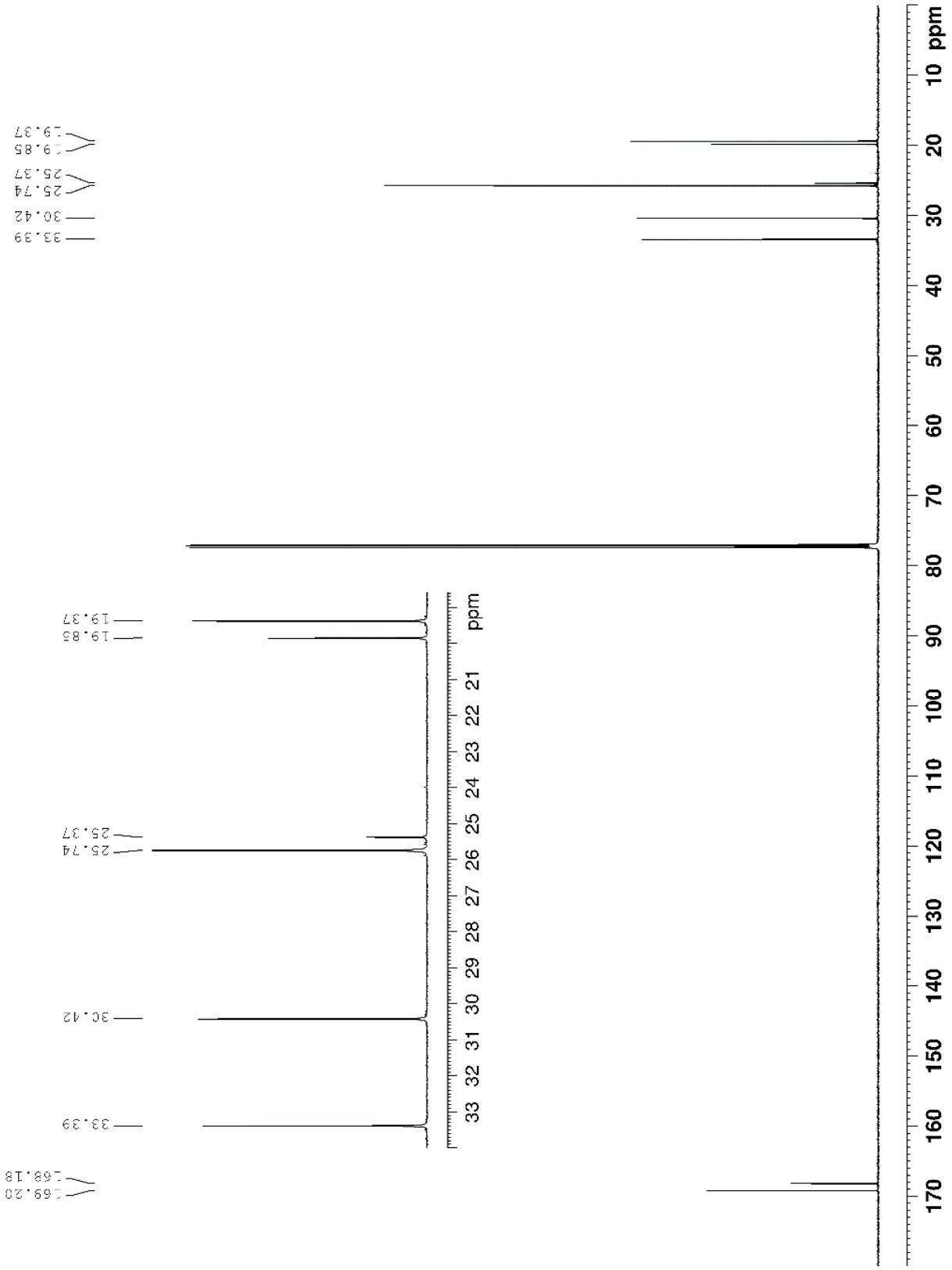
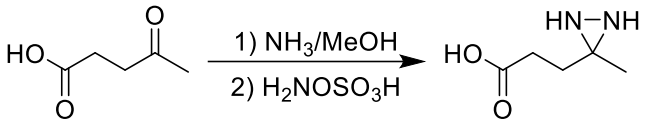


Figure 8: $^{13}C\{^{1}H\}$ NMR of 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate.

3.3 Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy} 2,5-dioxopyrrolidine-3-sulfonate

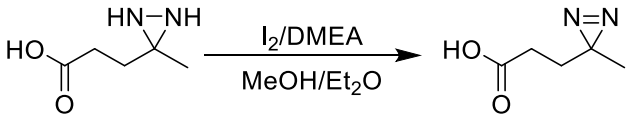
Certain elements of the procedure for the glassware synthesis of Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate were adapted from similar procedures found in literature.[1,2]

3.3.1 3-(3-methyldiaziridin-3-yl)propanoic acid



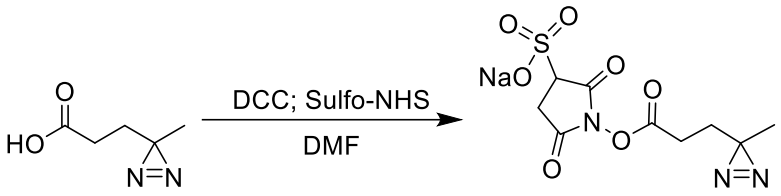
3-(3-methyldiaziridin-3-yl)propanoic acid was prepared as described previously in section 3.1.1

3.3.2 2.3.2 3-(3-methyl-3H-diazirin-3-yl)propanoic acid



3-(3-methyl-3H-diazirin-3-yl)propanoic acid was prepared as described previously in section 3.1.2

3.3.3 Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate



3-(3-methyl-3H-diazirin-3-yl)propanoic acid (0.331 g, 2.583 mmol) was dissolved in 10 mL of anhydrous DMF and stirred under argon in a 100 mL round bottom flask. N,N'-dicyclohexylmethanediimine (0.637 g, 3.087 mmol, 1.2 equiv) was then added into the vessel as a solid and the mixture stirred for 10 minutes. Sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate (0.558 g, 2.572 mmol, 1 equiv) was then added as a solid, the vessel closed with a rubber septum, evacuated and refilled with argon. The reaction mixture was placed in a water bath and stirred for 40 h at 25℃. The water bath was then replaced with an ice bath and the reaction stirred for 4 h. The cold suspension was filtered through grade 5 Whatman filter paper (pore size 2.5 μm), under continuous flow of dry nitrogen gas. The precipitate and the filter were quickly rinsed with 5 mL of freezer chilled, anhydrous DMF and discarded. Anhydrous ethyl acetate (80 mL) was then added into the filtrate, the mixture stirred briefly and left at 2℃ for 20 h in order to precipitate the product. Note that lower precipitation temperatures can result in lower purity product without noticeable increase in yield. The white precipitate of the product was filtered through grade 5 Whatman filter paper (pore size 2.5 μm), under continuous flow of dry nitrogen gas. Fresh anhydrous ethyl acetate (10 mL) was then added into the precipitation flask to recover any remaining precipitate in the form of suspension, this was also filtered through. 12 mL of fresh anhydrous ethyl acetate was then used directly to rinse the product on the filter, followed by 12 mL of anhydrous diethyl ether and 5 mL of anhydrous pentane. The filter paper along with the white precipitate was then dried overnight in a desiccator under vacuum. The dry product weighed 0.521 g, 1.591 mmol, giving yield 31% and purity 94%. ^{1}H NMR (600.1 MHz; 303 K; D_2O; δ, ppm; *J*, Hz): 4.52 (1H; bs), 3.42 (1H; bs), 3.22 (1H; dd; 2.9, 18.9), 2.73 (2H; t; 7.3), 1.86 (2H; t; 7.4), 1.09 (3H; s); ^{13}C{^{1}H} NMR (150.9 MHz, 303 K, D_2O; δ, ppm): 172.9, 172.2, 169.0, 59.4, 32.8, 31.4, 28.7, 28.2, 21.3.

Note that upon dissolution in D_2O the product begins to decompose, hence it is crucial to prepare NMR sample immediately before carrying out an NMR experiment. It was concluded that D_2O, despite this drawback, was superior in comparison with DMSO-d_6 since better peak separation could be achieved in both $^{13}C\{^1H\}$ and ^1H spectra.

3.3.4 NMR analysis of final product synthesized in glassware.

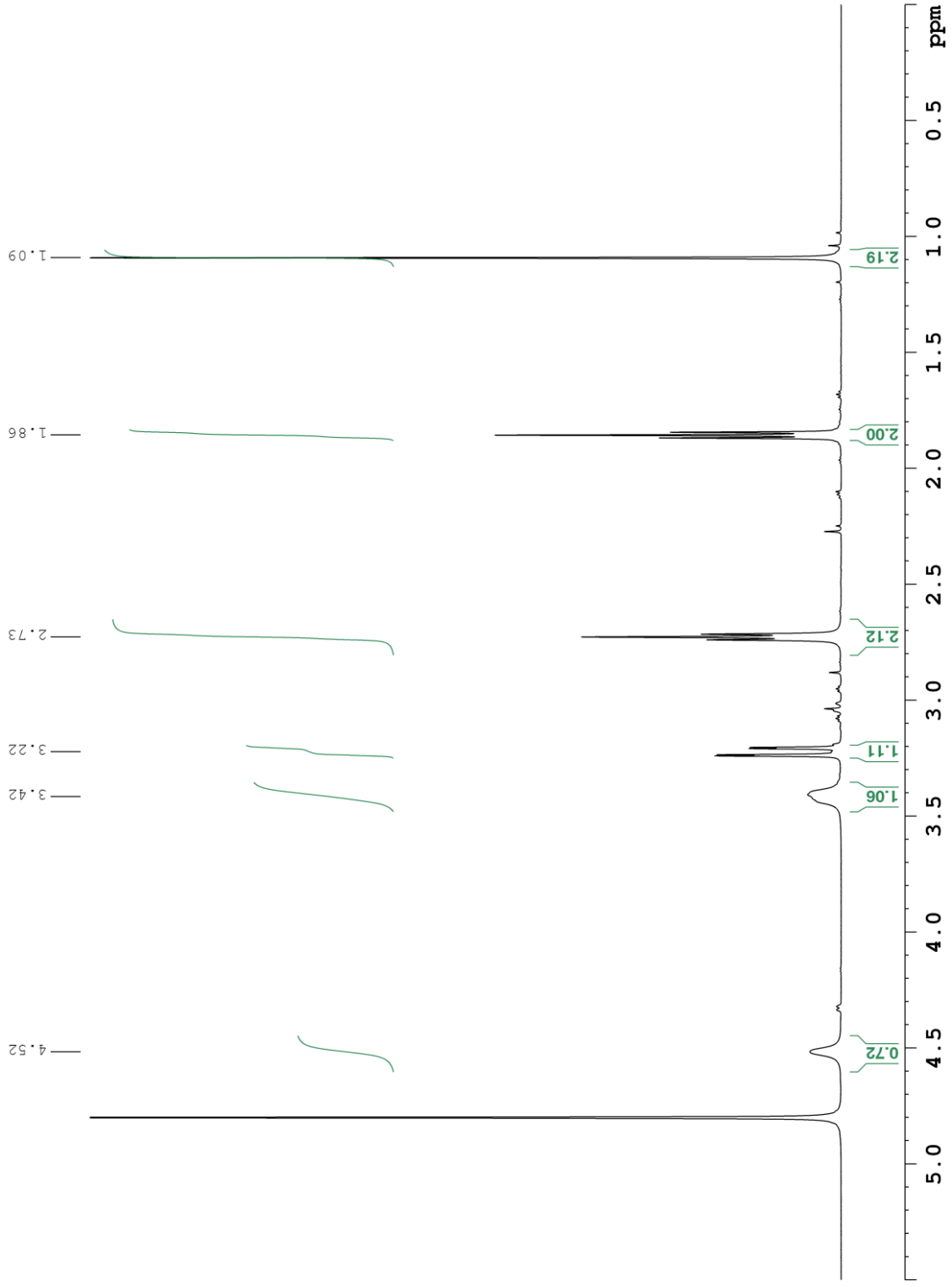


Figure 9: ^{1}H NMR of Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate.

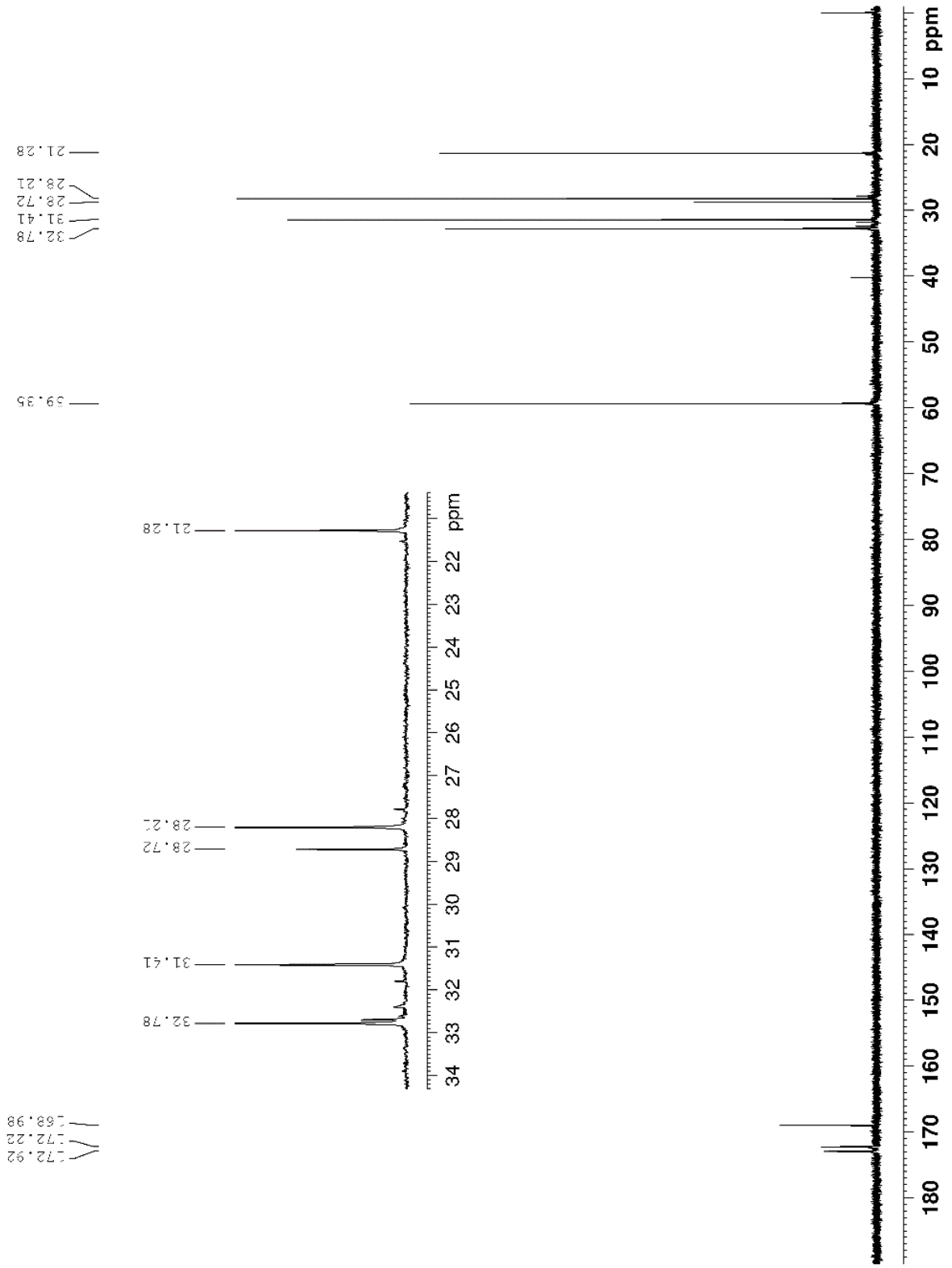
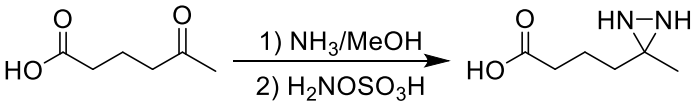


Figure 10: $^{13}C\{^{1}H\}$ NMR of Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate.

3.4 Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate

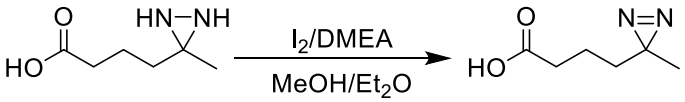
Certain elements of the procedure for the glassware synthesis of Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate were adapted from similar procedures found in literature.[1,2]

3.4.1 4-(3-methyldiaziridin-3-yl)butanoic acid



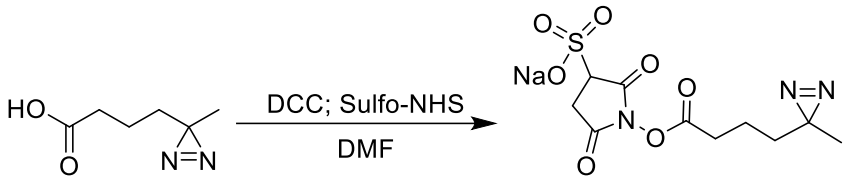
4-(3-methyldiaziridin-3-yl)butanoic acid was prepared as described previously in section 3.2.1

3.4.2 4-(3-methyl-3H-diazirin-3-yl)butanoic acid



4-(3-methyl-3H-diazirin-3-yl)butanoic acid was prepared as described previously in section 3.2.2

3.4.3 Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate



4-(3-methyl-3H-diazirin-3-yl)butanoic acid (0.294 g, 2.068 mmol) was dissolved in 10 mL of anhydrous DMF and stirred under argon in a 100 mL round bottom flask. N,N'-dicyclohexylmethanediimine (0.576 g, 2.787 mmol, 1.35 equiv) was then added into the vessel as solid and the mixture stirred for 10 minutes. Sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate (0.505 g, 2.326 mmol, 1.12 equiv) was then added as solid, the vessel closed with a rubber septum, vacuum evacuated and refilled with argon. The reaction mixture was placed in a water bath and stirred for 40 h at 25℃. The water bath was then replaced with an ice bath and the reaction stirred for 4 h. The cold suspension was filtered through grade 5 Whatman filter paper (pore size 2.5 μm), under continuous flow of dry nitrogen gas. The precipitate and the filter were rinsed with 5 mL of freezer chilled, anhydrous DMF and discarded. Anhydrous ethyl acetate (80 mL) was then added into the filtrate, the mixture stirred briefly and left at 2℃ for 20 h in order to precipitate the product. Note that lower precipitation temperatures can result in lower purity product without noticeable increase in yield. The white precipitate of the product was filtered through grade 5 Whatman filter paper (pore size 2.5 μm), under continuous flow of dry nitrogen gas. Fresh anhydrous ethyl acetate (10 mL) was then added into the precipitation flask to recover any remaining precipitate in the form of suspension, this was also filtered through. 12 mL of fresh anhydrous ethyl acetate was then used directly to rinse the product on the filter, followed by 12 mL of anhydrous diethyl ether and 5 mL of anhydrous pentane. The filter paper along with the white precipitate was then dried overnight in a desiccator under vacuum. The dried product weighed 0.507 g, 1.486 mmol, giving yield 29% and purity 97%. ^{1}H NMR (600.1 MHz; 303 K; D_2O; δ, ppm; *J*, Hz): 4.53 (1H, bs), 3.42 (1H; bs), 3.23 (1H; dd; 3.1, 18.9), 2.79 (2H; t; 7.3), 1.71 (2H; m), 1.52 (2H; t; 7.8), 1.08 (3H; s); ^{13}C{^{1}H} NMR (150.9 MHz, 303 K, D_2O; δ, ppm): 173.0, 172.7, 169.1, 59.4, 35.4, 32.8, 32.6, 29.3, 21.5, 21.4.

Note that upon dissolution in D_2O the product begins to decompose, hence it is crucial to prepare NMR sample immediately before carrying out NMR experiment. We found that D_2O, despite this drawback, was superior in comparison with DMSO-d_6 since better peak separation could be achieved in both $^{13}C\{^1H\}$ and ^1H spectra.

3.4.4 NMR analysis of final product synthesized in glassware.

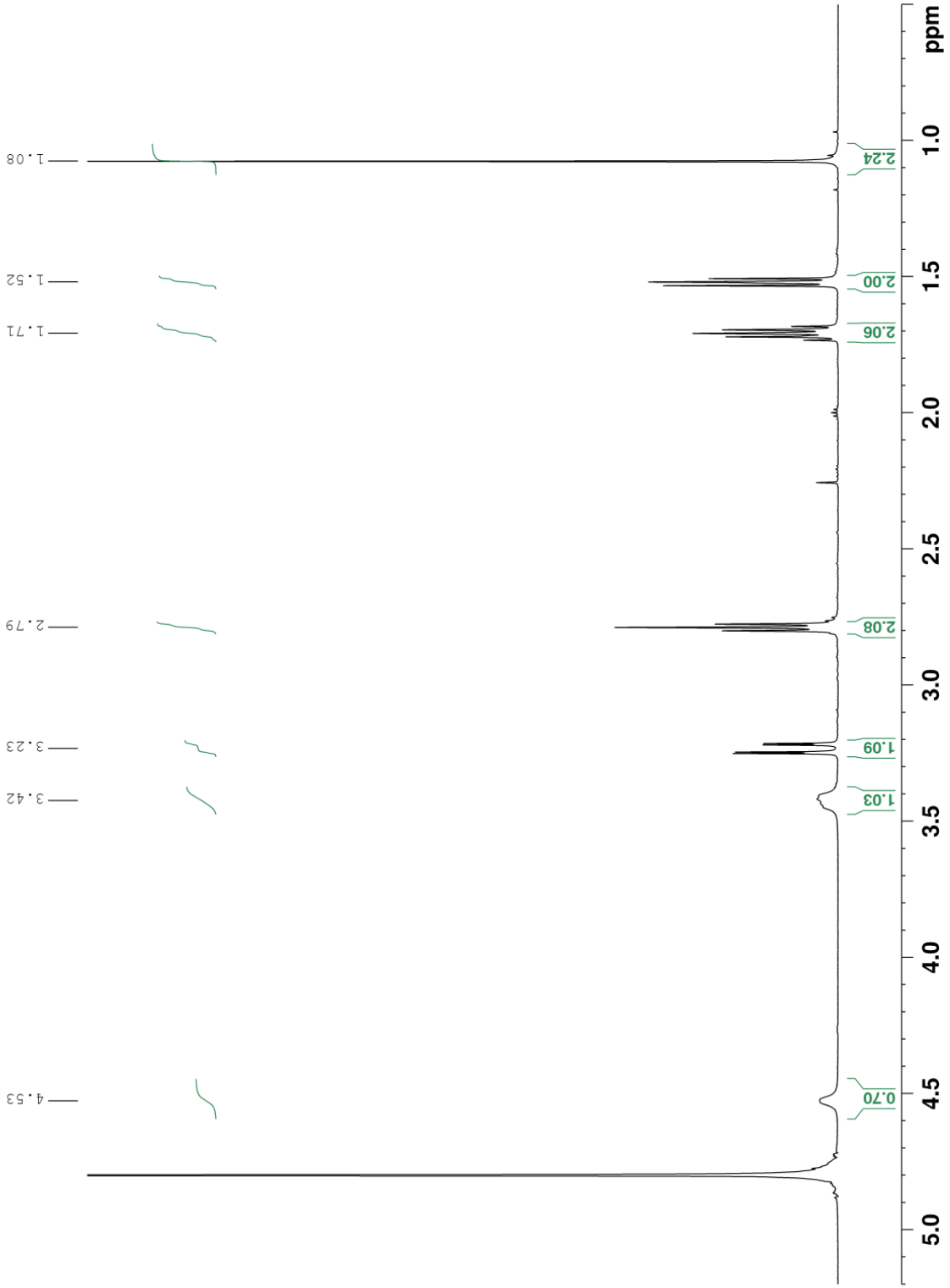


Figure 11: ^{1}H NMR of Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate

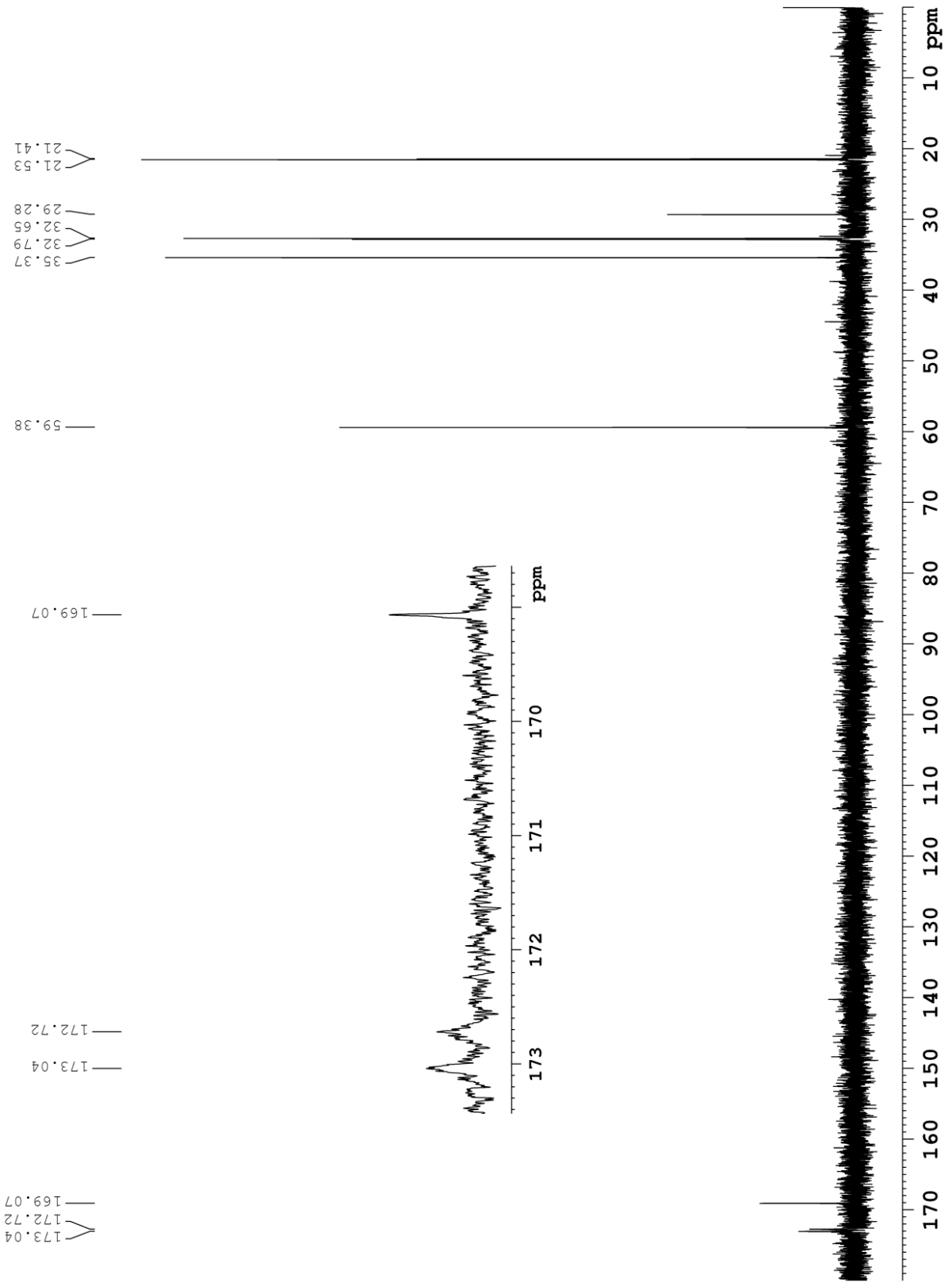


Figure 12: $^{13}C\{^{1}H\}$ NMR of Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate

4 Reactionware cartridge design and synthesis of target materials

4.1 General Remarks

All reactors used in this work were designed using OpenSCAD (http://www.openscad.org/) based software[3] and printed on Ultimaker 2+ 3D printers (https://ultimaker.com/), with 0.6 mm nozzles using polypropylene from a local supplier. The designs for the synthesis cartridges were exported as stereolithography (.stl) files and translated into 3D printer instruction files using Cura (https://ultimaker.com/en/products/cura-software), a freely available slicer software package developed by Ultimaker. The STL files provided as supplementary materials for the manuscript should be compatible with any suitable slicing software for 3D printers. These instruction files were then transferred to the 3D printer for fabrication. Devices were printed at 260°C using a layer height of 0.2 mm and a 3-layer raft extending 12 mm outside boundary of the models to avoid warping. The ultimaker base plate was exchanged for a 12 mm thick polypropylene plate to ensure adhesion of the print to the plate. As polypropylene is a non-standard material for 3D printing using the ultimaker device, it is likely that fabrication parameters will need to be tuned for individual 3D printers in order to produce reactor cartridges suitable for use. To allow for introduction of non-printed components such as fritted filter discs (**Figure 13, c**) and phase separators (**Figure 13, e**), the printing process was modified to pause at pre-programmed intervals during the fabrication to allow their placement. Once cartridge fabrication was complete the cartridges were flushed with a suitable inert gas (dry N_2 supplied by BOC) and sealed prior to use.

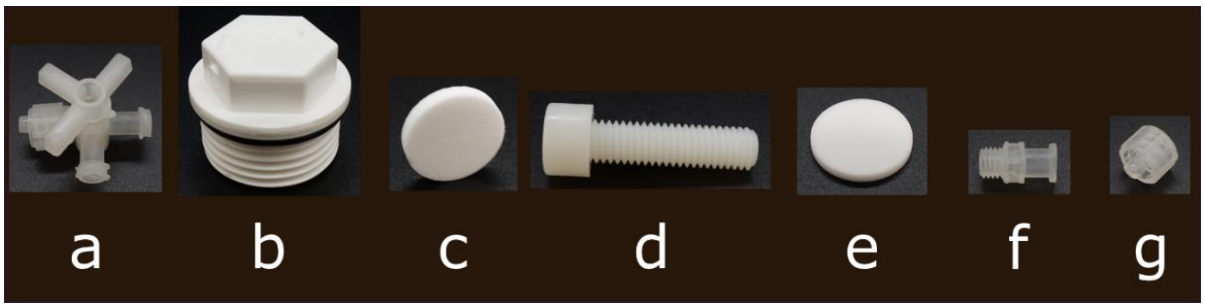


Figure 13. Non-printed parts: a) luer lock valve, b) module cap, c) fritted filter disc, d) screw valve, e) phase separator, f) luer to thread adapter, g) luer port cap

Figure 14. An example of a monolithic reactor fitted with non-printed elements

4.2 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate

To reduce the risk of any light induced decomposition throughout the synthesis, the modules were covered with aluminium foil whenever practical.

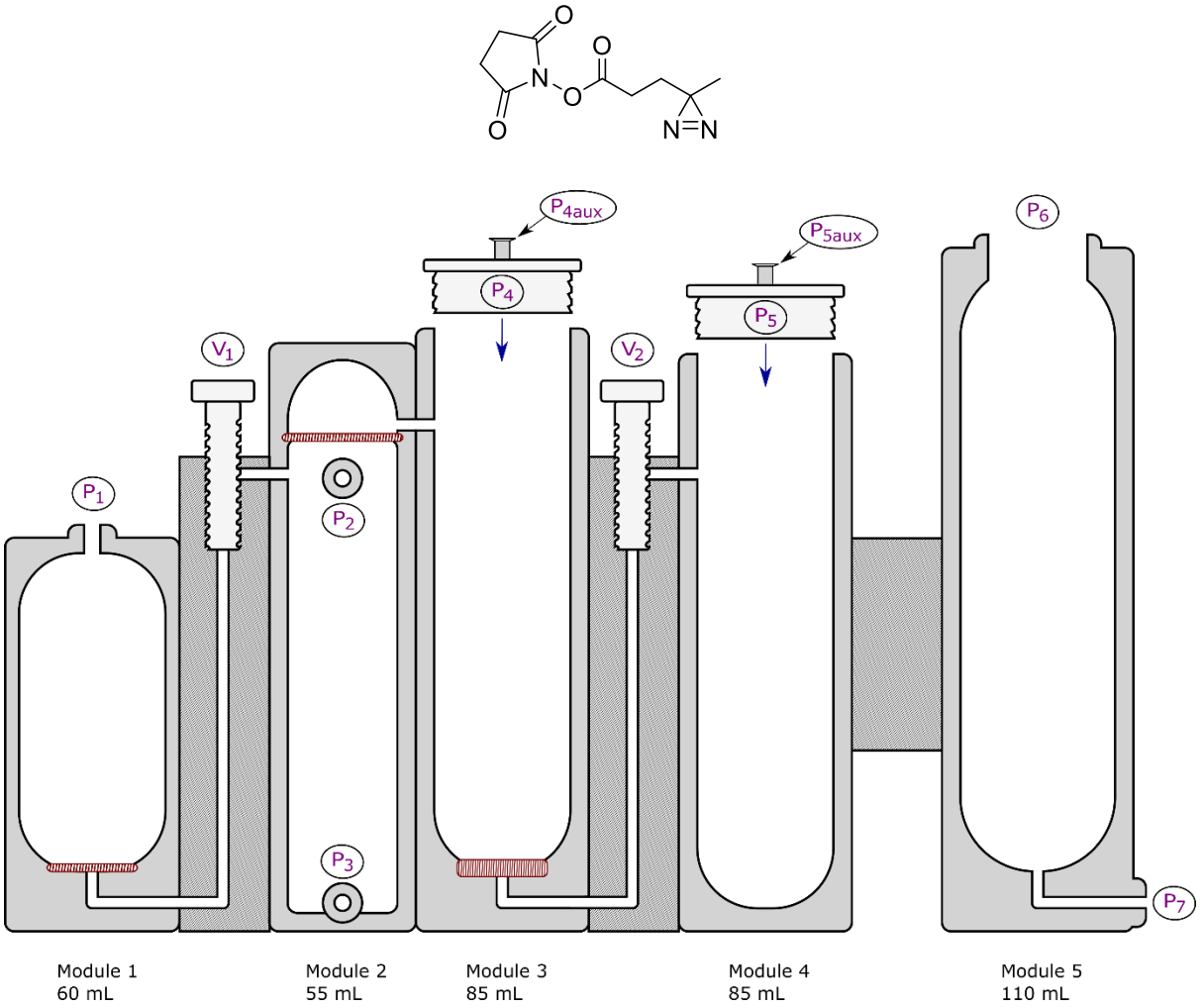


Figure 15: Schematic representation of cross section of the monolithic reactor used in syntheses of NHS-diazirines

4.2.1 Synthetic procedure for reactionware.

Levulinic acid (0.60 g, 5.167 mmol) dissolved in 1.5 mL of anhydrous methanol was added into the module 1, through port P_1. The reaction vessel was placed in a cooling bath at 0°C. 7M solution of ammonia in methanol (9 ml, 63 mmol, 12.2 equiv) was then added into module 1 and the mixture stirred under nitrogen atmosphere for 1 h. The module was opened briefly to add 1.1 g of dried magnesium sulfate. The mixture was stirred for additional 5 h at 0°C.

Hydroxylamine-O-sulfonic acid (0.701 g, 6.204 mmol, 1.2 equiv) was dissolved in 5.5 mL of anhydrous MeOH and added into the reaction module dropwise via syringe, over a course of 5 min. The reaction was then stirred for additional 16 h at 0℃. The cooling was stopped and the reaction mixture transferred to module 2 by applying gentle inert gas pressure to port P_1. The solvent was then evaporated by means of vacuum connected to port P_2. Note that evaporation was carried out at 20℃ to avoid decomposition and at vacuum not exceeding 50 mbar to avoid loss of the intermediate via evaporation. Following removal of the solvent and ammonia, module 2 was flushed with argon and the crude of diaziridine re-dissolved in 7 mL of anhydrous MeOH introduced via port P_2. The reaction monolith was placed inside an ice bath again and N,N-dimethylethylamine (0.57 mL, 5.26 mmol, 1.02 equiv) was introduced, through port P_2. This was stirred for 3 min, after which gradual addition of iodine beads commenced. When adding the iodine, the colour of the reaction mixture became brown initially, but within a few seconds the colour disappeared to give a colourless solution again. Upon further addition of iodine, the rate of the disappearance of the brown colour slowed significantly. When the brown colour faded within 10 min of the last addition giving yellow tinted solution at the end of the 10 min period, the addition of iodine was stopped. Note that 0.811 g, 3.195 mmol, 0.618 equiv of iodine was normally required. The reaction was then left to stir for another 30 min. The ice bath was then removed and 3 mL of aqueous KI solution (50% w/v) was introduced into the module, through port P_2. This was followed by 1.2 mL of 50% w/v aqueous solution of ascorbic acid and finally 1 mL of 3M HCl, added dropwise. The mixture was extracted immediately with 70 ml of diethyl ether introduced via port P_3. Finally, 35 mL of saturated solution of sodium chloride was also introduced to enhance the recovery of the organic phase from the reaction module. The remaining contents of module 2 were then withdrawn via port P_3 and discarded. Note that upon addition of the extraction solvent and the sodium chloride solution to module 2, the organic phase containing the product flowed into module 3 preloaded with vacuum dried (at 120℃) $MgSO_4$. The extract was dried overnight at ambient temperature. The following day, the organic extract was transferred to module 4 by means of gentle inert gas pressure applied to port P_{4aux} of module 3. Once transferred, vacuum was applied to port P_{5aux} in order to evaporate the solvent. Note that care must be taken during evaporation of the solvent as high vacuum applied for extended periods of time will cause loss of yield due to evaporation of the product itself. For this reason,

evaporation was generally carried out at 20-25°C and vacuum not exceeding 50 mbar. Once completed, the vacuum supply was cut off and module 4 was filled with argon. Anhydrous acetonitrile (1.5 mL) of was introduced through port P_5 to solubilize the crude diazirine acid. The cartridge monolith was then cooled by means of an ice bath. N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide (0.562 g, 3.620 mmol) was dissolved in 3 mL of anhydrous DCM and introduced into the module, followed by *N*-hydroxysuccinimide (0.417 g, 3.620 mmol) dissolved in 3 mL of anhydrous acetonitrile. The module was closed and the mixture stirred on an ice bath for further 30 min. The cooling was then removed and a few grains of freshly dried 3Å molecular sieves were added into the reaction vessel. The monolith was sealed and placed in a water bath set to 25°C, the reaction was then left to stir for another 18 h. The reaction mixture was concentrated by partial evaporation of the solvent at 25°C and max 100 mbar vacuum, leaving approximately 2 mL of brown liquid visible on the bottom of the module. The crude was transferred into module 5 and purified by silica column chromatography using a mixture of anhydrous PET 40-60 and anhydrous ethyl acetate in 70:30 ratio, respectively. Note that the silica was thoroughly dried prior to use and loaded into column as a suspension in the eluent.* To aid the transfer of the crude from module 4 to chromatography module 5, additional 2 mL of anhydrous DCM was used. Collection of the eluent from the column began immediately after loading of the crude. Passage of eluent through the column may be aided by a small amount of positive pressure of inert gas. 268 mL of eluent was collected and discarded first. Following 282 mL of eluent was collected and evaporated, leaving behind the product as white solid with a final mass of 0.454 g, 2.016 mmol, yield 39% and 94% purity. Note that the eluent was collected and evaporated outside of the cartridge. ^{1}H NMR (600.1 MHz; 303 K; $CDCl_3$; δ, ppm; *J*, Hz): 2.84 (4H, bs), 2.52 (2H; t; 7.8), 1.81 (2H; t; 7.8), 1.07 (3H; s); $^{13}C\{^{1}H\}$ NMR (150.9 MHz, 303 K, $CDCl_3$; δ, ppm): 169.1, 167.8, 29.7, 25.9, 25.7, 24.9, 19.7.

***Note on preparation of the column chromatography module.** First, the eluent outflow channel in the bottom of the chromatography module was loosely packed with cotton wool and the base of the module covered with 8 mm of oven dried sand. The module was then filled with silica, carefully introduced as suspension in eluent as not to disturb the even bed of sand on the bottom of the module. The height of the settled silica in the column was 75 mm, measured from the top of the sand bed to the top of the settled surface of silica. Note

that the silica was dried at 100-120℃ under vacuum (~10 mbar) for period of 3-4h. Subsequently it was allowed to return to room temperature and stored under argon, prior to use. Solvents used in the eluent mixture were dried thoroughly by means of 3Å molecular sieves. Molecular sieves were dried before use at 200℃ in vacuum (5 mbar) for a period of at least 3 h. The dried sieves were stored under argon, prior to use. The eluent composition was 70:30 ratio of PET40-60 and ethyl acetate, respectively.

4.2.2 Design and optimization of the cartridge geometry

Initially, each step in the synthetic process requiring a designated module was carried out in such a module as a discrete unit. This strategy was adopted to ensure that each module performed as required and intermediate outcomes in the synthesis were of satisfactory quality. Once optimal design parameters were resolved, the modules were being gradually connected together and tested for their performance as part of an assembly. Following this approach, the entire system of modules was developed and optimised to best fulfil its purpose.

Since the entire synthetic process was enclosed in monolithic system formed out of five modules it was necessary to ensure that no undesired transfer of a reaction mixture was possible between neighbouring units. To ensure this, screw valves were fitted to the siphons located between the vessels. The valves were normally closed, until it became necessary to transfer reaction content from one unit to another. Semitransparency of the polypropylene modules generally allowed to gauge a level of liquid and its colour inside a module. Whenever this was not sufficient, the required area of a module was backlit with a low power LED torch (Ansmann T50F, 60 lumens).

4.2.3 Operations sequence

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
1	0/00:00 +00:01	Add Levulinic acid (0.6 g) dissolved in 1.5 mL of anhydrous methanol, to module 1, through port P_1	O	C	C	C	C	C	C	C	C
2	0/00:01 +00:01	Place the cartridge in a cooling bath at 0°C	O	C	C	C	C	C	C	C	C
3	0/00:02 +00:04	Add 9 mL of 7M ammonia in methanol, through port P_1	O	C	C	C	C	C	C	C	C
4	0/00:06 +00:01	Connect a nitrogen filled balloon to P_1	O N_2	C	C	C	C	C	C	C	C
5	0/00:07 +01:00	Stir for 1 h	O N_2	C	C	C	C	C	C	C	C
6	0/01:07 +00:01	Disconnect the balloon from P_1	O	C	C	C	C	C	C	C	C
7	0/01:08 +00:02	Add 1.1 g of dried $MgSO_4$, through port P_1	O	C	C	C	C	C	C	C	C
8	0/01:10 +00:01	Connect nitrogen filled balloon to P_1	O N_2	C	C	C	C	C	C	C	C
9	0/01:11 +05:00	Stir for 5 h at 0°C	O N_2	C	C	C	C	C	C	C	C
10	0/06:11 +00:01	Disconnect the balloon from P_1	O	C	C	C	C	C	C	C	C
11	0/06:12 +00:05	Add dropwise a solution of hydroxylamine-O-sulfonic acid (0.701 g) dissolved in 5.5 mL of dry methanol, via a syringe connected to port P_1	O	C	C	C	C	C	C	C	C
12	0/06:17 +00:01	Close port P_1	C	C	C	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
13	0/06:18 +16:00	Stir for 16 h at 0°C	C	C	C	C	C	C	C	C	C
14	0/22:18 +00:01	Remove the cooling	C	C	C	C	C	C	C	C	C
15	0/22:19 +00:01	Open port P_1	O	C	C	C	C	C	C	C	C
16	0/22:20 +00:02	Open valve V_1 to enable transfer of liquid from module 1 to module 2	O	O	C	C	C	C	C	C	C
17	0/22:22 +00:01	Open port P_2	O	O	O	C	C	C	C	C	C
18	0/22:23 +00:00	Stir at 250 rpm	O	O	O	C	C	C	C	C	C
19	0/22:23 +00:10	Apply gentle inert gas pressure to port P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C
20	0/22:33 +00:01	Add 2 mL of dry methanol to module 1, through P_1	O	O	O	C	C	C	C	C	C
21	0/22:34 +00:05	Apply gentle inert gas pressure to P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C
22	0/22:39 +00:01	Add another 2 mL of dry methanol to module 1, through P_1	O	O	O	C	C	C	C	C	C
23	0/22:40 +00:05	Apply gentle inert gas pressure to P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C
24	0/22:45 +00:01	Close P_1	C	O	O	C	C	C	C	C	C
25	0/22:46 +00:02	Close valve V_1	C	C	O	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
26	0/22:48 +06:00	Apply vacuum [note 1] to P_2 to partially evaporate the solvent in module 2. Reduce the volume of the liquid to approximately 2 mL	C	C	O Vac	C	C	C	C	C	C
27	1/04:48 +00:02	Close the vacuum and connect a source of argon to port P_2. Re-equilibrate the pressure inside module 2. Leave connected to the argon source.	C	C	O Arg	C	C	C	C	C	C
28	1/05:00 +00:01	Place the monolith in a cooling bath at 0°C	C	C	O Arg	C	C	C	C	C	C
29	1/05:01 +00:05	Wait 5 min	C	C	O Arg	C	C	C	C	C	C
30	1/05:06 +00:01	Disconnect the argon source	C	C	O	C	C	C	C	C	C
31	1/05:07 +00:01	Add 7 mL of dry methanol through P_2	C	C	O	C	C	C	C	C	C
32	1/05:08 +00:01	Stir for 1 min	C	C	O	C	C	C	C	C	C
33	1/05:09 +00:01	Add 0.57 mL of N,N-dimethylethylamine through port P_2	C	C	O	C	C	C	C	C	C
34	1/05:10 +00:03	Stir for 3 min	C	C	O	C	C	C	C	C	C
35	1/05:13 +02:00	Add I_2 in small portions as solid through port P_2 [note 2] Maintain stirring at approximately 300 rpm	C	C	O	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
36	1/07:13 +00:30	Stir for additional 30 min after the last addition of I_2	C	C	C	C	C	C	C	C	C
37	1/07:43 +00:01	Remove the cooling	C	C	C	C	C	C	C	C	C
38	1/07:44 +00:01	Stir at 250 rpm and add 3 mL of aqueous KI solution (50 % w/v), through P_2.	C	C	O	C	C	C	C	C	C
39	1/07:45 +00:01	Add 1.2 mL of 50 % w/v aqueous solution of ascorbic acid, through P_2.	C	C	O	C	C	C	C	C	C
40	1/07:46 +00:02	Increase the rate of stirring to approximately 400 rpm. Use port P_2 to add 1 mL of 3M HCl, dropwise over a course of 2 min.	C	C	O	C	C	C	C	C	C
41	1/07:48 +00:01	Close port P_2	C	C	C	C	C	C	C	C	C
42	1/07:49 +00:00	Stop stirring	C	C	C	C	C	C	C	C	C
43	1/07:49 +00:03	Open port P_4 and add 3 g dried magnesium sulfate to module 3	C	C	C	C	O	C	C	C	C
44	1/07:52 +00:03	Close P_4 and open P_4aux	C	C	C	C	O Aux	C	C	C	C
45	1/07:55 +00:02	Introduce 20 mL of diethyl ether through valve at port P_3	C	C	C	O Solv	O Aux	C	C	C	C
46	1/07:57 +00:00	Stir for 30 sec at 350 rpm	C	C	C	O Solv	O Aux	C	C	C	C
47	1/07:57 +00:01	Stop stirring	C	C	C	O Solv	O Aux	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
48	1/07:58 +00:02	Introduce 10 mL of diethyl ether through valve at port P_3	C	C	C	O Solv	O Aux	C	C	C	C
49	1/08:00 +00:00	Stir for 30 sec at 350 rpm	C	C	C	O Solv	O Aux	C	C	C	C
50	1/08:00 +00:01	Stop stirring	C	C	C	O Solv	O Aux	C	C	C	C
51	1/08:01 +00:12	Repeat operations 48 – 50 four more times	C	C	C	O Solv	O Aux	C	C	C	C
52	1/08:13 +00:05	Ensure the stirring if off. Slowly introduce 35 mL of saturated aqueous sodium chloride solution through port P_3	C	C	C	O Sol	O Aux	C	C	C	C
53	1/08:18 +00:03	Withdraw the waste liquid from module 2 through P_3	C	C	C	O	O Aux	C	C	C	C
54	1/08:21 +00:01	Close ports P_3 and P_4aux	C	C	C	C	C	C	C	C	C
55	1/08:22 +16:00	Place the cartridge in a dark place at ambient temperature, to allow for drying of the organic extract	C	C	C	C	C	C	C	C	C
56	2/00:22 +00:03	Open ports P_4aux and P_5aux. Open valve V_2	C	C	C	C	O Aux	O	O Aux	C	C
57	2/00:25 +00:05	Apply gentle inert gas pressure to P_4aux and transfer the liquid from module 3 to module 4	C	C	C	C	O Aux	O	O Aux	C	C
58	2/00:30 +00:02	Open port P_4 and add 5 mL of dry diethyl ether into module 3 [note 3]	C	C	C	C	O	O	O Aux	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
59	2/00:32 +00:02	Close P_4	C	C	C	C	O Aux	O	O Aux	C	C
60	2/00:34 +00:03	Apply gentle inert gas pressure to P_4aux and transfer the liquid from module 3 to module 4	C	C	C	C	O Aux	O	O Aux	C	C
61	2/00:37 +00:14	Repeat operations 58 – 60 two more times	C	C	C	C	O/ Aux	O	O Aux	C	C
62	2/00:51 +00:03	Close port P_4aux and valve V_2	C	C	C	C	C	C	O Aux	C	C
63	2/00:53 +00:00	Start stirring at 250 rpm	C	C	C	C	C	C	O Aux	C	C
64	2/00:53 +06:00	Apply vacuum to port P_5aux and evaporate the solvent from module 4 note 4	C	C	C	C	C	C	O Aux -Vac	C	C
65	2/06:53 +00:02	Once completed, close the vacuum and fill module 4 with argon	C	C	C	C	C	C	O Aux -Arg	C	C
66	2/06:55 +00:04	Open port P_5 and add 1.5 mL of anh acetonitrile to module 4 note 5	C	C	C	C	C	C	O	C	C
67	2/06:59 +00:02	Close port P_5	C	C	C	C	C	C	C	C	C
68	2/07:01 +00:02	Stir for 1 min then place the cartridge in an ice bath at 0°C	C	C	C	C	C	C	C	C	C
69	2/07:03 +00:01	Open port P_5aux	C	C	C	C	C	C	O Aux	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
70	2/07:04 +00:02	Add N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide (0.562 g) solubilised in 3 mL of anhydrous DCM	C	C	C	C	C	C	O Aux	C	C
71	2/07:06 +00:02	Add *N*-hydroxysuccinimide (0.417 g) dissolved in 3 mL of anhydrous acetonitrile	C	C	C	C	C	C	O Aux	C	C
72	2/07:08 +00:01	Close port P_5aux	C	C	C	C	C	C	C	C	C
73	2/07:09 +00:30	Stir for 30 min	C	C	C	C	C	C	C	C	C
74	2/07:39 +00:02	Remove cooling. Open port P_5 and add 10-15 grains of freshly dried 3Å molecular sieves	C	C	C	C	C	C	O	C	C
75	2/07:41 +00:01	Close port P_5	C	C	C	C	C	C	C	C	C
76	2/07:42 +00:03	Place the monolith in a water bath. Set temperature of the bath to 25°C	C	C	C	C	C	C	C	C	C
77	2/07:45 +18:00	Stir for further 18 h	C	C	C	C	C	C	C	C	C
78	3/01:45 +04:00	Connect vacuum to port P_5aux and partially evaporate the solvent note 6	C	C	C	C	C	C	O Aux -Vac	C	C
79	3/05:45 +00:02	Once completed, close the vacuum and fill module 4 with argon	C	C	C	C	C	C	O Aux -Arg	C	C
80	3/05:47 +00:30	Prepare the column chromatography module note 7	C	C	C	C	C	C	O Aux -Vac	O	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
81	3/06:17 +00:03	Use a glass pipette to transfer the crude from module 4 onto the surface of the silica in module 5. Ensure even distribution across the surface of the silica.	C	C	C	C	C	C	O	O	C
82	3/06:20 +00:01	Open port P_7 and carefully release a small volume of the eluent in order to bring the surface of the liquid flush with the surface of silica	C	C	C	C	C	C	O	O	O
83	3/06:21 +00:01	Close port P_7	C	C	C	C	C	C	O	O	C
84	3/06:22 +00:01	Add 2 mL of anhydrous DCM into module 4 and stir briefly	C	C	C	C	C	C	O	O	C
85	3/06:23 +00:01	Transfer the liquid onto the surface of the silica in module 5	C	C	C	C	C	C	O	O	C
86	3/06:24 +00:02	Repeat step 82-83	C	C	C	C	C	C	O	O	O/C
87	3/06:26 +00:01	Deposit 1.5 mL of the eluent mixture onto the surface of the silica	C	C	C	C	C	C	O	O	C
88	3/06:27 +00:01	Open port P_7 and begin to collect the eluent immediately	C	C	C	C	C	C	C	O	O
89	3/06:28 +00:01	Using a glass pipette, carefully begin to deposit the eluent onto the surface of the silica [note 8]	C	C	C	C	C	C	C	O	O
90	3/06:29 +01:00	Collect and discard the first 268 mL of eluent	C	C	C	C	C	C	C	O	O

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
91	3/07:29 +01:03	Collect the following 282 mL of eluent	C	C	C	C	C	C	C	O	O
92	3/08:32	Evaporate the eluent note 9	C	C	C	C	C	C	C	C	C
93		Store the purified product at -18°C, under argon, away from light.									

note 1: Note that evaporation was carried out at 20°C to avoid decomposition and at vacuum not exceeding 50 mbar to avoid loss of the intermediate via evaporation.

note 2: When adding the iodine, the colour of the reaction mixture becomes brown initially, but within a few seconds the colour disappears to give a colourless solution again. Upon further addition of iodine, the rate of the disappearance of the brown colour slows significantly. When the brown colour fades within 10 min of the last addition giving yellow tinted solution at the end of the 10 min period, the addition of iodine should be stopped.

note 3: Use syringe. Use the solvent to wash down any crude that may have deposited on the walls of the module.

note 4: Initially use gentle vacuum (~900 mbar) to avoid a boil-off of the solvent and loss of the organic extract into the vacuum line. Over time gradually increase the power of the vacuum. Note that care must be taken during evaporation of the solvent as high vacuum applied for extended periods of time will cause loss of yield due to evaporation of the product itself. For this reason, evaporation was generally carried out at 20-25°C and vacuum not exceeding 50 mbar.

note 5: Use syringe. Use the solvent to wash down any crude that may have deposited on the walls of the module during evaporation.

note 6: Concentrate the reaction mixture by evaporating of the solvent at 25°C and 100 mbar vacuum, leaving approximately 2 mL of brown liquid visible on the bottom of the module. Use gentle vacuum initially, to avoid boil-off of the solvent and loss of the crude.

note 7: First loosely pack cotton wool in the eluent outflow channel in the bottom of the chromatography module. Cover the base of the module with 8 mm of oven dried sand. Carefully introduce silica suspended in eluent, making sure that the even bed of the sand on the bottom of the module is not disturbed. The height of settled silica in the column should be 75 mm, measured from the top of the sand bed to the top of the settled surface of silica. Note that the silica should be dried at 100-120°C under vacuum (~10 mbar) for period of 3-4h, then cooled down to room temperature and stored under argon, prior to use. Thoroughly

dry the solvents used in the eluent mixture by means of 3A molecular sieves. Dry the molecular sieves at 200°C under strong vacuum for a period of at least 3 h. Store under argon prior to use. Prepare the eluent mixture by mixing PET 40-60 and ethyl acetate in 70:30 ratio. Ensure that the surface of the eluent is flush with the surface of the silica inside the chromatography module, prior to deposition of the crude onto the surface of the silica.

note 8: Initially deposit approximately 1 mL of eluent per 15 seconds, just enough to keep the level of solvent flush with the silica surface. Repeat this process till approximately 25 mL of eluent is used. Following that, carefully increase the rate of the solvent addition till the solvent level is just below the brim of the module. To maintain a good rate of flow of the eluent at port P_7, attach B24 rubber septum, fitted with a balloon filled with argon, to port P_6.

note 9: Carry out the evaporation outside of the reactionware modules.

4.2.4 NMR analysis of product synthesized in reactionware.

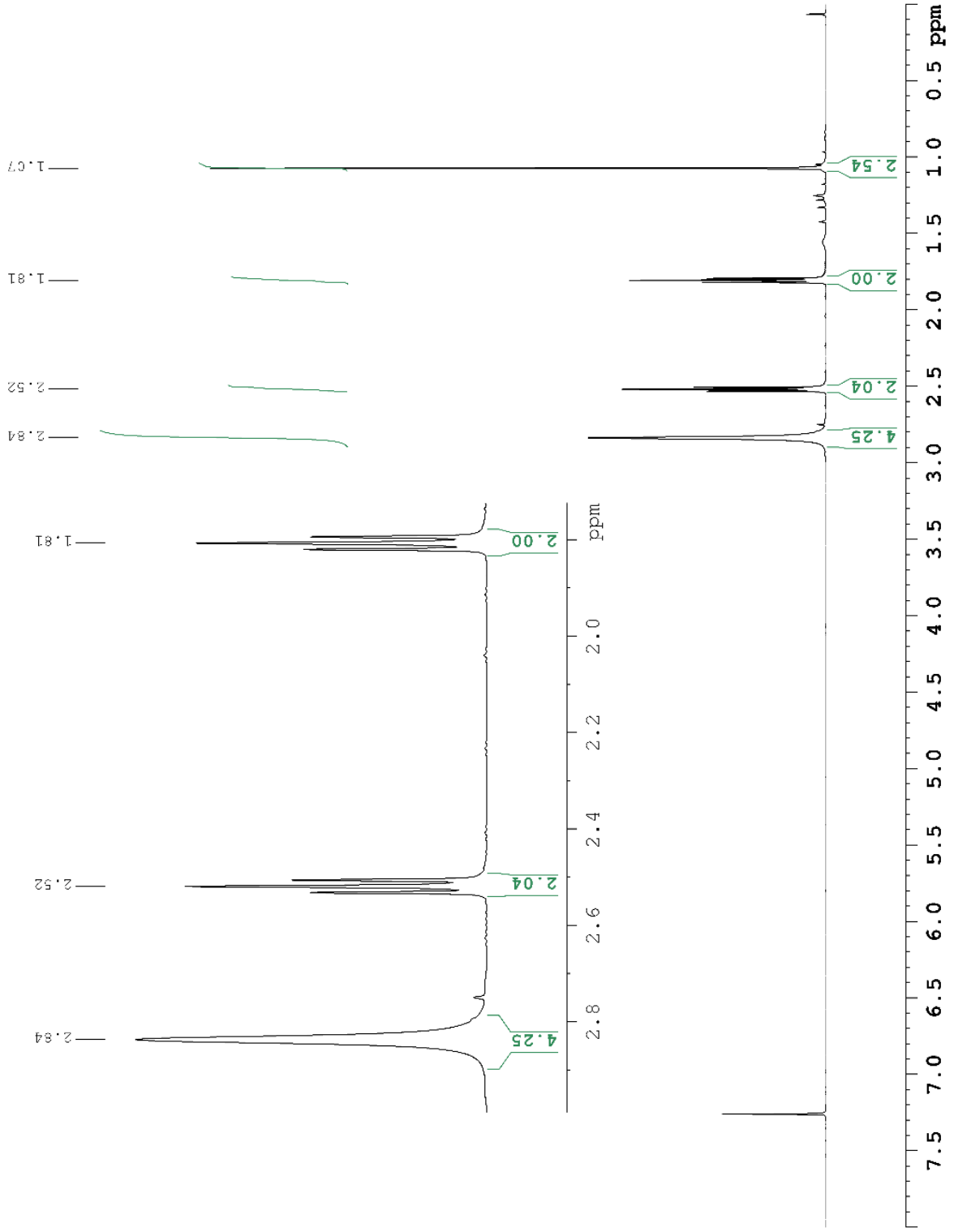


Figure 16: ^{1}H NMR of 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate.

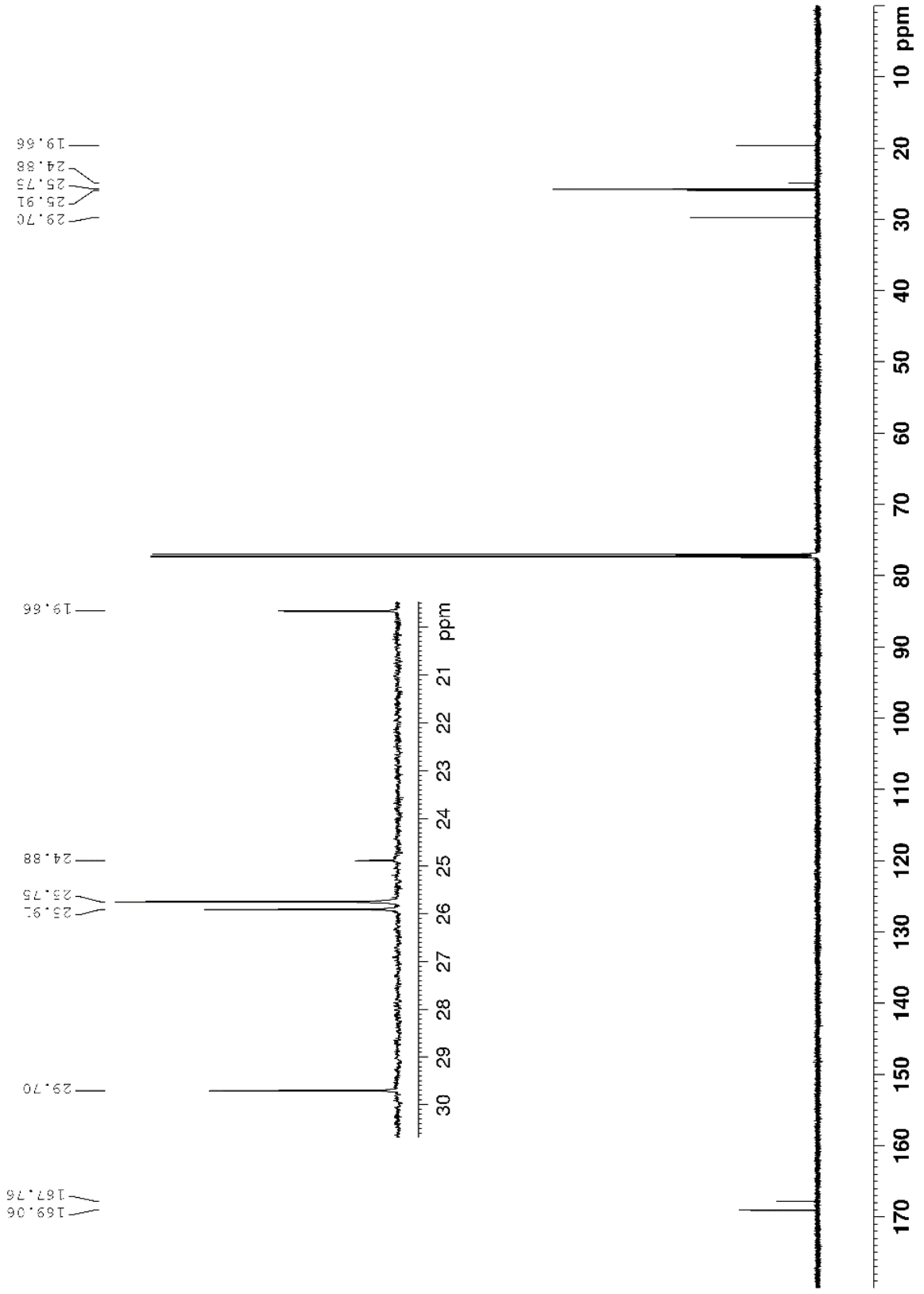


Figure 17: $^{13}C\{^{1}H\}$ NMR of 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate.

4.3 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate

To reduce the risk of any light induced decomposition throughout the synthesis, the modules were covered with aluminium foil whenever practical.

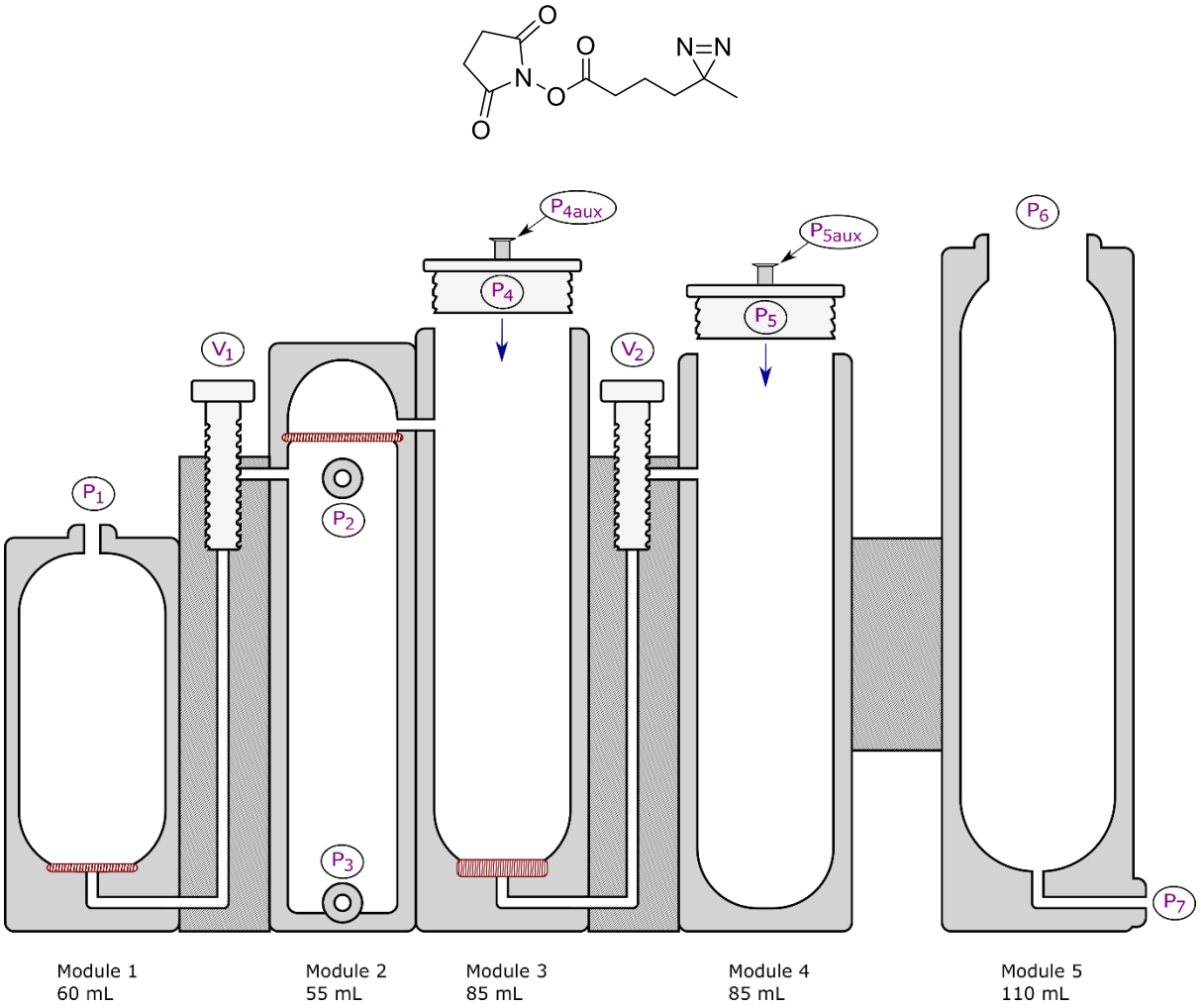


Figure 18: Schematic representation of cross section of the monolithic reactor used in syntheses of NHS-diazirines

4.3.1 Synthetic procedure for reactionware.

4-Acetylbutyric acid (0.673 g, 5.17 mmol) dissolved in 2 mL of anhydrous methanol was added into the module 1, through port P_1. The reaction vessel was placed in a cooling bath at 0°C. 7M solution of ammonia in methanol (9 ml, 63 mmol, 12.2 equiv) was then added into module 1 and the mixture stirred under nitrogen atmosphere for 1 h. The module was opened briefly to add 1.1 g of dried magnesium sulfate. The mixture was stirred for additional 5 h at 0°C.

Hydroxylamine-O-sulfonic acid (0.701 g, 6.204 mmol, 1.2 equiv) was dissolved in 5.5 mL of anhydrous MeOH and added into the reaction module dropwise via syringe, over a course of 5 min. The reaction was then stirred for additional 16 h at 0℃. The cooling was stopped and the reaction mixture transferred to module 2 by applying gentle inert gas pressure to port P_1. The solvent was then evaporated by means of vacuum connected to port P_2. Module 2 was then flushed with argon and the crude of diaziridine re-dissolved in 7 mL of anhydrous MeOH introduced via port P_2. The reaction monolith was placed inside an ice bath again and N,N-dimethylethylamine (0.57 mL, 5.26 mmol, 1.02 equiv) was introduced, through port P_2. This was stirred for 3 min, after which gradual addition of iodine beads commenced. When adding the iodine, the colour of the reaction mixture became brown initially, but within a few seconds the colour disappeared to give a colourless solution again. Upon further addition of iodine, the rate of the disappearance of the brown colour slowed significantly. When the brown colour faded within 10 min of the last addition giving yellow tinted solution at the end of the 10 min period, the addition of iodine was stopped. Note that 0.743 g, 2.927 mmol, 0.57 equiv of iodine was normally required. The reaction was then left to stir for another 30 min. The ice bath was then removed and 3 mL of aqueous KI solution (50% w/v) was introduced into the module, through port P_2. This was followed by 1.2 mL of 50% w/v aqueous solution of ascorbic acid and finally 1 mL of 3M HCl, added dropwise. The mixture was extracted immediately with 70 ml of diethyl ether introduced via port P_3. Finally, 35 mL of saturated solution of sodium chloride was also introduced to enhance the recovery of the organic phase from the reaction module. The remaining contents of module 2 were then withdrawn via port P_3 and discarded. Note that upon addition of the extraction solvent and the sodium chloride solution to module 2, the organic phase containing the product flowed into module 3 preloaded with vacuum dried (at 120℃) $MgSO_4$. The extract was dried overnight at ambient temperature. The following day, the organic extract was transferred to module 4 by means of gentle inert gas pressure applied to port P_{4aux} of module 3. Once transferred, vacuum was applied to port P_{5aux} in order to evaporate the solvent. Once completed, the vacuum supply was cut off and module 4 was filled with argon. Anhydrous acetonitrile (1.5 mL) of was introduced through port P_5 to solubilize the crude diazirine acid. The cartridge monolith was then cooled by means of an ice bath. N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide (0.562 g, 3.620 mmol) was dissolved in 3 mL of anhydrous DCM and introduced into the module,

followed by *N*-hydroxysuccinimide (0.417 g, 3.620 mmol) dissolved in 3 mL of anhydrous acetonitrile. The module was closed and the mixture stirred on an ice bath for further 30 min. The cooling was then removed and a few grains of freshly dried 3Å molecular sieves were added into the reaction vessel. The monolith was sealed and placed in a water bath set to 25℃, the reaction was then left to stir for another 18 h. The reaction mixture was concentrated by partial evaporation of the solvent at 25℃ and max 100 mbar vacuum, leaving approximately 2 mL of brown liquid visible on the bottom of the module. The crude was transferred into module 5 and purified by silica column chromatography using a mixture of anhydrous PET 40-60 and anhydrous ethyl acetate in 75:25 ratio, respectively. Note that the silica was thoroughly dried prior to use and loaded into column as a suspension in the eluent.* To aid the transfer of the crude from module 4 to chromatography module 5, additional 2 mL of anhydrous DCM was used. Collection of the eluent from the column began immediately after loading of the crude. 382 mL of eluent was collected and discarded first. Following 352 mL of eluent was collected and evaporated, leaving behind the product as clear, off-yellow liquid. Passage of eluent through the column may be aided by a small amount of positive pressure of inert gas. Note that the eluent was collected and evaporated outside of the cartridge. After initial evaporation of the eluent, the product contained traces of low boiling impurities originating from PET 40-60. To mitigate this the product was re-dissolved in 3 mL of deuterated chloroform and the solvent thoroughly evaporated again, giving material with a final mass of 0.372 g, 1.555 mmol, yield 30% and 97% purity. ^{1}H NMR (600.1 MHz; 303 K; $CDCl_3$; δ, ppm; *J*, Hz): 2.83 (4H, bs), 2.60 (2H; t; 7.3), 1.64 (2H; m), 1.48 (2H; t; 7.9), 1.03 (3H; s); $^{13}C\{^{1}H\}$ NMR (150.9 MHz, 303 K, $CDCl_3$; δ, ppm): 169.2, 168.2, 33.4, 30.4, 25.7, 25.4, 19.8, 19.4.

***Note on preparation of the column chromatography module.** First, the eluent outflow channel in the bottom of the chromatography module was loosely packed with cotton wool and the base of the module covered with 8 mm of oven dried sand. The module was then filled with silica, carefully introduced as suspension in eluent as not to disturb the even bed of sand on the bottom of the module. The height of the settled silica in the column was 75 mm, measured from the top of the sand bed to the top of the settled surface of silica. Note that the silica was dried at 100-120℃ under vacuum (~10 mbar) for period of 3-4h. Subsequently it was allowed to return to room temperature and stored under argon, prior to

use. Solvents used in the eluent mixture were dried thoroughly by means of 3Å molecular sieves. Molecular sieves were dried before use at 200°C in vacuum (5 mbar) for a period of at least 3 h. The dried sieves were stored under argon, prior to use. The eluent composition was 75:25 ratio of PET40-60 and ethyl acetate, respectively.

4.3.2 Design and optimization of the cartridge geometry

Initially, each step in the synthetic process requiring a designated module was carried out in such a module as a discrete unit. This strategy was adopted to ensure that each module performed as required and intermediate outcomes in the synthesis were of satisfactory quality. Once optimal design parameters were resolved, the modules were being gradually connected together and tested for their performance as part of an assembly. Following this approach, the entire system of modules was developed and optimised to best fulfil its purpose.

Since the entire synthetic process was enclosed in monolithic system formed out of five modules it was necessary to ensure that no undesired transfer of a reaction mixture was possible between neighbouring units. To ensure this, screw valves were fitted to the siphons located between the vessels. The valves were normally closed, until it became necessary to transfer reaction content from one unit to another. Semitransparency of the polypropylene modules generally allowed to gauge a level of liquid and its colour inside a module. Whenever this was not sufficient, the required area of a module was backlit with a low power LED torch (Ansmann T50F, 60 lumens).

4.3.3 Operations sequence

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
1	0/00:00 +00:01	Add 4-acetylbutyric acid (0.673 g) dissolved in 2 mL of anhydrous methanol, to module 1, through port P_1	O	C	C	C	C	C	C	C	C
2	0/00:01 +00:01	Place the cartridge in a cooling bath at 0°C	O	C	C	C	C	C	C	C	C
3	0/00:02 +00:04	Add 9 mL of 7M ammonia in methanol, through port P_1	O	C	C	C	C	C	C	C	C
4	0/00:06 +00:01	Connect a nitrogen filled balloon to P_1	O N_2	C	C	C	C	C	C	C	C
5	0/00:07 +01:00	Stir for 1 h	O N_2	C	C	C	C	C	C	C	C
6	0/01:07 +00:01	Disconnect the balloon from P_1	O	C	C	C	C	C	C	C	C
7	0/01:08 +00:02	Add 1.1 g of dried $MgSO_4$, through port P_1	O	C	C	C	C	C	C	C	C
8	0/01:10 +00:01	Connect nitrogen filled balloon to P_1	O N_2	C	C	C	C	C	C	C	C
9	0/01:11 +05:00	Stir for 5 h at 0°C	O N_2	C	C	C	C	C	C	C	C
10	0/06:11 +00:01	Disconnect the balloon from P_1	O	C	C	C	C	C	C	C	C
11	0/06:12 +00:05	Add dropwise a solution of hydroxylamine-O-sulfonic acid (0.701 g) dissolved in 5.5 mL of dry methanol, via a syringe connected to port P_1	O	C	C	C	C	C	C	C	C
12	0/06:17 +00:01	Close port P_1	C	C	C	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
13	0/06:18 +16:00	Stir for 16 h at 0°C	C	C	C	C	C	C	C	C	C
14	0/22:18 +00:01	Remove the cooling	C	C	C	C	C	C	C	C	C
15	0/22:19 +00:01	Open port P_1	O	C	C	C	C	C	C	C	C
16	0/22:20 +00:02	Open valve V_1 to enable transfer of liquid from module 1 to module 2	O	O	C	C	C	C	C	C	C
17	0/22:22 +00:01	Open port P_2	O	O	O	C	C	C	C	C	C
18	0/22:23 +00:00	Stir at 250 rpm	O	O	O	C	C	C	C	C	C
19	0/22:23 +00:10	Apply gentle inert gas pressure to port P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C
20	0/22:33 +00:01	Add 2 mL of dry methanol to module 1, through P_1	O	O	O	C	C	C	C	C	C
21	0/22:34 +00:05	Apply gentle inert gas pressure to P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C
22	0/22:39 +00:01	Add another 2 mL of dry methanol to module 1, through P_1	O	O	O	C	C	C	C	C	C
23	0/22:40 +00:05	Apply gentle inert gas pressure to P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C
24	0/22:45 +00:01	Close P_1	C	O	O	C	C	C	C	C	C
25	0/22:46 +00:02	Close valve V_1	C	C	O	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
26	0/22:48 +06:00	Apply vacuum [note 1] to P_2 to partially evaporate the solvent in module 2. Reduce the volume of the liquid to approximately 2 mL	C	C	O Vac	C	C	C	C	C	C
27	1/04:48 +00:02	Close the vacuum and connect a source of argon to port P_2. Re-equilibrate the pressure inside module 2. Leave connected to the argon source.	C	C	O Arg	C	C	C	C	C	C
28	1/05:00 +00:01	Place the monolith in a cooling bath at 0°C	C	C	O Arg	C	C	C	C	C	C
29	1/05:01 +00:05	Wait 5 min	C	C	O Arg	C	C	C	C	C	C
30	1/05:06 +00:01	Disconnect the argon source	C	C	O	C	C	C	C	C	C
31	1/05:07 +00:01	Add 7 mL of dry methanol through P_2	C	C	O	C	C	C	C	C	C
32	1/05:08 +00:01	Stir for 1 min	C	C	O	C	C	C	C	C	C
33	1/05:09 +00:01	Add 0.57 mL of N,N-dimethylethylamine through port P_2	C	C	O	C	C	C	C	C	C
34	1/05:10 +00:03	Stir for 3 min	C	C	O	C	C	C	C	C	C
35	1/05:13 +02:00	Add I_2 in small portions as solid through port P_2 [note 2] Maintain stirring at approximately 300 rpm	C	C	O	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
36	1/07:13 +00:30	Stir for additional 30 min after the last addition of I_2	C	C	C	C	C	C	C	C	C
37	1/07:43 +00:01	Remove the cooling	C	C	C	C	C	C	C	C	C
38	1/07:44 +00:01	Stir at 250 rpm and add 3 mL of aqueous KI solution (50 % w/v), through P_2.	C	C	O	C	C	C	C	C	C
39	1/07:45 +00:01	Add 1.2 mL of 50 % w/v aqueous solution of ascorbic acid, through P_2.	C	C	O	C	C	C	C	C	C
40	1/07:46 +00:02	Increase the rate of stirring to approximately 400 rpm. Use port P_2 to add 1 mL of 3M HCl, dropwise over a course of 2 min.	C	C	O	C	C	C	C	C	C
41	1/07:48 +00:01	Close port P_2	C	C	C	C	C	C	C	C	C
42	1/07:49 +00:00	Stop stirring	C	C	C	C	C	C	C	C	C
43	1/07:49 +00:03	Open port P_4 and add 3 g dried magnesium sulfate to module 3	C	C	C	C	O	C	C	C	C
44	1/07:52 +00:03	Close P_4 and open P_4aux	C	C	C	C	O Aux	C	C	C	C
45	1/07:55 +00:02	Introduce 20 mL of diethyl ether through valve at port P_3	C	C	C	O Solv	O Aux	C	C	C	C
46	1/07:57 +00:00	Stir for 30 sec at 350 rpm	C	C	C	O Solv	O Aux	C	C	C	C
47	1/07:57 +00:01	Stop stirring	C	C	C	O Solv	O Aux	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
48	1/07:58 +00:02	Introduce 10 mL of diethyl ether through valve at port P_3	C	C	C	O Solv	O Aux	C	C	C	C
49	1/08:00 +00:00	Stir for 30 sec at 350 rpm	C	C	C	O Solv	O Aux	C	C	C	C
50	1/08:00 +00:01	Stop stirring	C	C	C	O Solv	O Aux	C	C	C	C
51	1/08:01 +00:12	Repeat operations 48 – 50 four more times	C	C	C	O Solv	O Aux	C	C	C	C
52	1/08:13 +00:05	Ensure the stirring if off. Slowly introduce 35 mL of saturated aqueous sodium chloride solution through port P_3	C	C	C	O Sol	O Aux	C	C	C	C
53	1/08:18 +00:03	Withdraw the waste liquid from module 2 through P_3	C	C	C	O	O Aux	C	C	C	C
54	1/08:21 +00:01	Close ports P_3 and P_4aux	C	C	C	C	C	C	C	C	C
55	1/08:22 +16:00	Place the cartridge in a dark place at ambient temperature, to allow for drying of the organic extract	C	C	C	C	C	C	C	C	C
56	2/00:22 +00:03	Open ports P_4aux and P_5aux. Open valve V_2	C	C	C	C	O Aux	O	O Aux	C	C
57	2/00:25 +00:05	Apply gentle inert gas pressure to P_4aux and transfer the liquid from module 3 to module 4	C	C	C	C	O Aux	O	O Aux	C	C
58	2/00:30 +00:02	Open port P_4 and add 5 mL of dry diethyl ether into module 3 [note 3]	C	C	C	C	O	O	O Aux	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
59	2/00:32 +00:02	Close P_4	C	C	C	C	O Aux	O	O Aux	C	C
60	2/00:34 +00:03	Apply gentle inert gas pressure to P_4aux and transfer the liquid from module 3 to module 4	C	C	C	C	O Aux	O	O Aux	C	C
61	2/00:37 +00:14	Repeat operations 58 – 60 two more times	C	C	C	C	O/ Aux	O	O Aux	C	C
62	2/00:51 +00:03	Close port P_4aux and valve V_2	C	C	C	C	C	C	O Aux	C	C
63	2/00:53 +00:00	Start stirring at 250 rpm	C	C	C	C	C	C	O Aux	C	C
64	2/00:53 +06:00	Apply vacuum to port P_5aux and evaporate the solvent from module 4 note 4	C	C	C	C	C	C	O Aux -Vac	C	C
65	2/06:53 +00:02	Once completed, close the vacuum and fill module 4 with argon	C	C	C	C	C	C	O Aux -Arg	C	C
66	2/06:55 +00:04	Open port P_5 and add 1.5 mL of anh acetonitrile to module 4 note 5	C	C	C	C	C	C	O	C	C
67	2/06:59 +00:02	Close port P_5	C	C	C	C	C	C	C	C	C
68	2/07:01 +00:02	Stir for 1 min then place the cartridge in an ice bath at 0°C	C	C	C	C	C	C	C	C	C
69	2/07:03 +00:01	Open port P_5aux	C	C	C	C	C	C	O Aux	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
70	2/07:04 +00:02	Add N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide (0.562 g) solubilised in 3 mL of anhydrous DCM	C	C	C	C	C	C	O Aux	C	C
71	2/07:06 +00:02	Add *N*-hydroxysuccinimide (0.417 g) dissolved in 3 mL of anhydrous acetonitrile	C	C	C	C	C	C	O Aux	C	C
72	2/07:08 +00:01	Close port P_5aux	C	C	C	C	C	C	C	C	C
73	2/07:09 +00:30	Stir for 30 min	C	C	C	C	C	C	C	C	C
74	2/07:39 +00:02	Remove cooling. Open port P_5 and add 10-15 grains of freshly dried 3Å molecular sieves	C	C	C	C	C	C	O	C	C
75	2/07:41 +00:01	Close port P_5	C	C	C	C	C	C	C	C	C
76	2/07:42 +00:03	Place the monolith in a water bath. Set temperature of the bath to 25°C	C	C	C	C	C	C	C	C	C
77	2/07:45 +18:00	Stir for further 18 h	C	C	C	C	C	C	C	C	C
78	3/01:45 +04:00	Connect vacuum to port P_5aux and partially evaporate the solvent [note 6]	C	C	C	C	C	C	O Aux -Vac	C	C
79	3/05:45 +00:02	Once completed, close the vacuum and fill module 4 with argon	C	C	C	C	C	C	O Aux -Arg	C	C
80	3/05:47 +00:30	Prepare the column chromatography module [note 7]	C	C	C	C	C	C	O Aux -Vac	O	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
81	3/06:17 +00:03	Use a glass pipette to transfer the crude from module 4 onto the surface of the silica in module 5. Ensure even distribution across the surface of the silica.	C	C	C	C	C	C	O	O	C
82	3/06:20 +00:01	Open port P_7 and carefully release a small volume of the eluent in order to bring the surface of the liquid flush with the surface of silica	C	C	C	C	C	C	O	O	O
83	3/06:21 +00:01	Close port P_7	C	C	C	C	C	C	O	O	C
84	3/06:22 +00:01	Add 2 mL of anhydrous DCM into module 4 and stir briefly	C	C	C	C	C	C	O	O	C
85	3/06:23 +00:01	Transfer the liquid onto the surface of the silica in module 5	C	C	C	C	C	C	O	O	C
86	3/06:24 +00:02	Repeat step 82-83	C	C	C	C	C	C	O	O	O/C
87	3/06:26 +00:01	Deposit 1.5 mL of the eluent mixture onto the surface of the silica	C	C	C	C	C	C	O	O	C
88	3/06:27 +00:01	Open port P_7 and begin to collect the eluent immediately	C	C	C	C	C	C	C	O	O
89	3/06:28 +00:01	Using a glass pipette, carefully begin to deposit the eluent onto the surface of the silica[note 8]	C	C	C	C	C	C	C	O	O
90	3/06:29 +01:21	Collect and discard the first 382 mL of eluent	C	C	C	C	C	C	C	O	O

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7
91	3/07:50 +01:15	Collect the following 352 mL of eluent	C	C	C	C	C	C	C	O	O
92	3/09:05	Evaporate the eluent note 9	C	C	C	C	C	C	C	C	C
93		Store the purified product at -18°C, under argon, away from light.									

note 1: Note that evaporation was carried out at 20°C.

note 2: When adding the iodine, the colour of the reaction mixture becomes brown initially, but within a few seconds the colour disappears to give a colourless solution again. Upon further addition of iodine, the rate of the disappearance of the brown colour slows significantly. When the brown colour fades within 10 min of the last addition giving yellow tinted solution at the end of the 10 min period, the addition of iodine should be stopped.

note 3: Use syringe. Use the solvent to wash down any crude that may have deposited on the walls of the module.

note 4: Initially use gentle vacuum (~900 mbar) to avoid a boil-off of the solvent and loss of the organic extract into the vacuum line. Over time gradually increase the power of the vacuum.

note 5: Use syringe. Use the solvent to wash down any crude that may have deposited on the walls of the module during evaporation.

note 6: Concentrate the reaction mixture by evaporating of the solvent at 25°C and 100 mbar vacuum, leaving approximately 2 mL of brown liquid visible on the bottom of the module. Use gentle vacuum initially, to avoid boil-off of the solvent and loss of the crude.

note 7: First loosely pack cotton wool in the eluent outflow channel in the bottom of the chromatography module. Cover the base of the module with 8 mm of oven dried sand. Carefully introduce silica suspended in eluent, making sure that the even bed of the sand on the bottom of the module is not disturbed. The height of settled silica in the column should be 75 mm, measured from the top of the sand bed to the top of the settled surface of silica. Note that the silica should be dried at 100-120°C under vacuum (~10 mbar) for period of 3-4h, then cooled down to room temperature and stored under argon, prior to use. Thoroughly dry the solvents used in the eluent mixture by means of 3A molecular sieves. Dry the molecular sieves at 200°C under strong vacuum for a period of at least 3 h. Store under argon prior to use. Prepare the eluent mixture by mixing PET 40-60 and ethyl acetate in 75:25 ratio.

Ensure that the surface of the eluent is flush with the surface of the silica inside the chromatography module, prior to deposition of the crude onto the surface of the silica.

note 8: Initially deposit approximately 1 mL of eluent per 15 seconds, just enough to keep the level of solvent flush with the silica surface. Repeat this process till approximately 25 mL of eluent is used. Following that, carefully increase the rate of the solvent addition till the solvent level is just below the brim of the module. To maintain a good rate of flow of the eluent at port P_7, attach B24 rubber septum, fitted with a balloon filled with argon, to port P_6.

note 9: Carry out the evaporation outside of the reactionware modules. Note that after initial evaporation of the eluent, the product can contain traces of low boiling point impurities originating from Pet 40-60. To mitigate this, re-dissolve the product in 3 mL of deuterated chloroform and carry out another evaporation in order to obtain the pure product.

4.3.4 NMR analysis of product synthesized in reactionware.

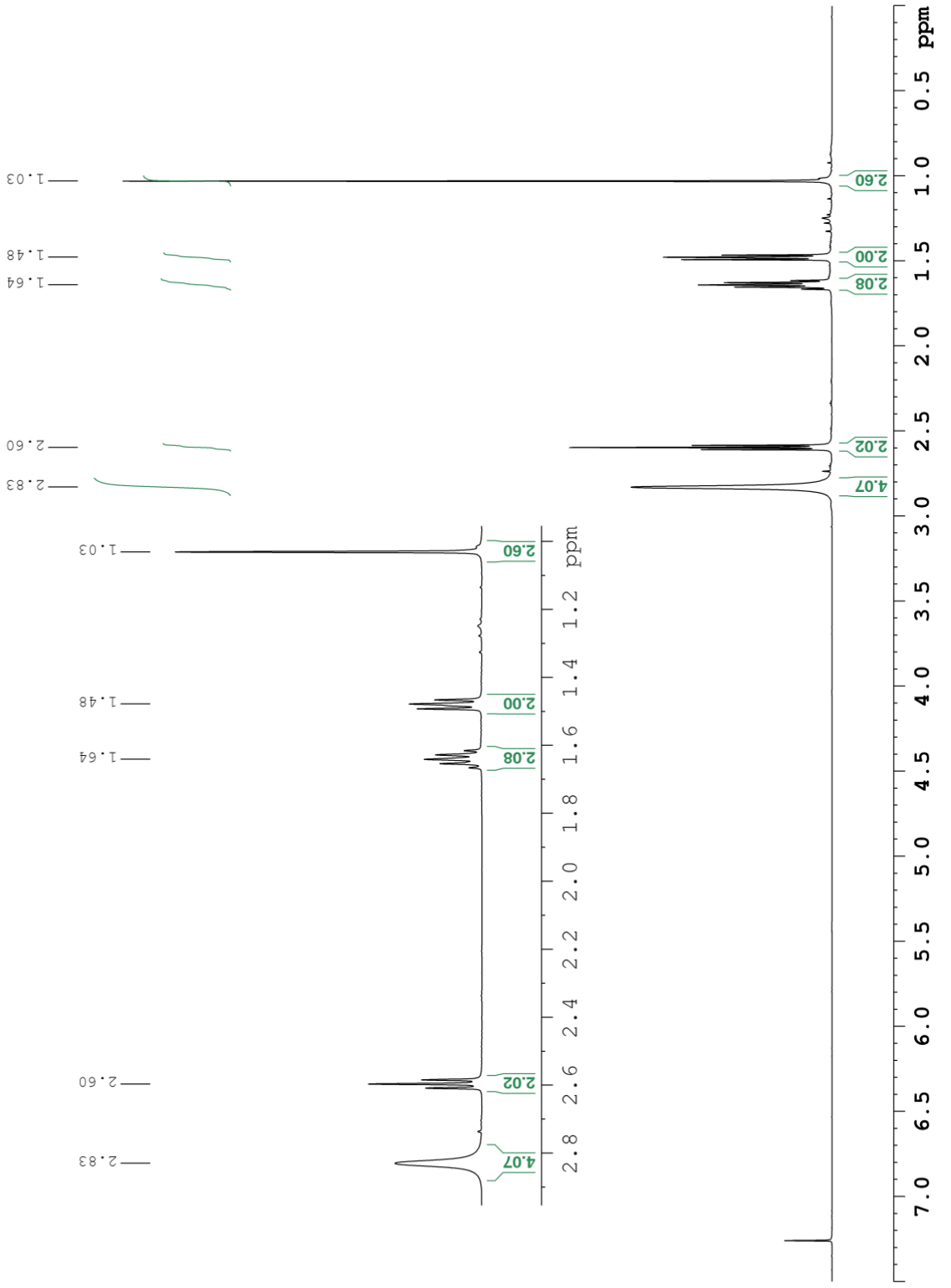


Figure 19: ^{1}H NMR of 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate synthesized in reactionware.

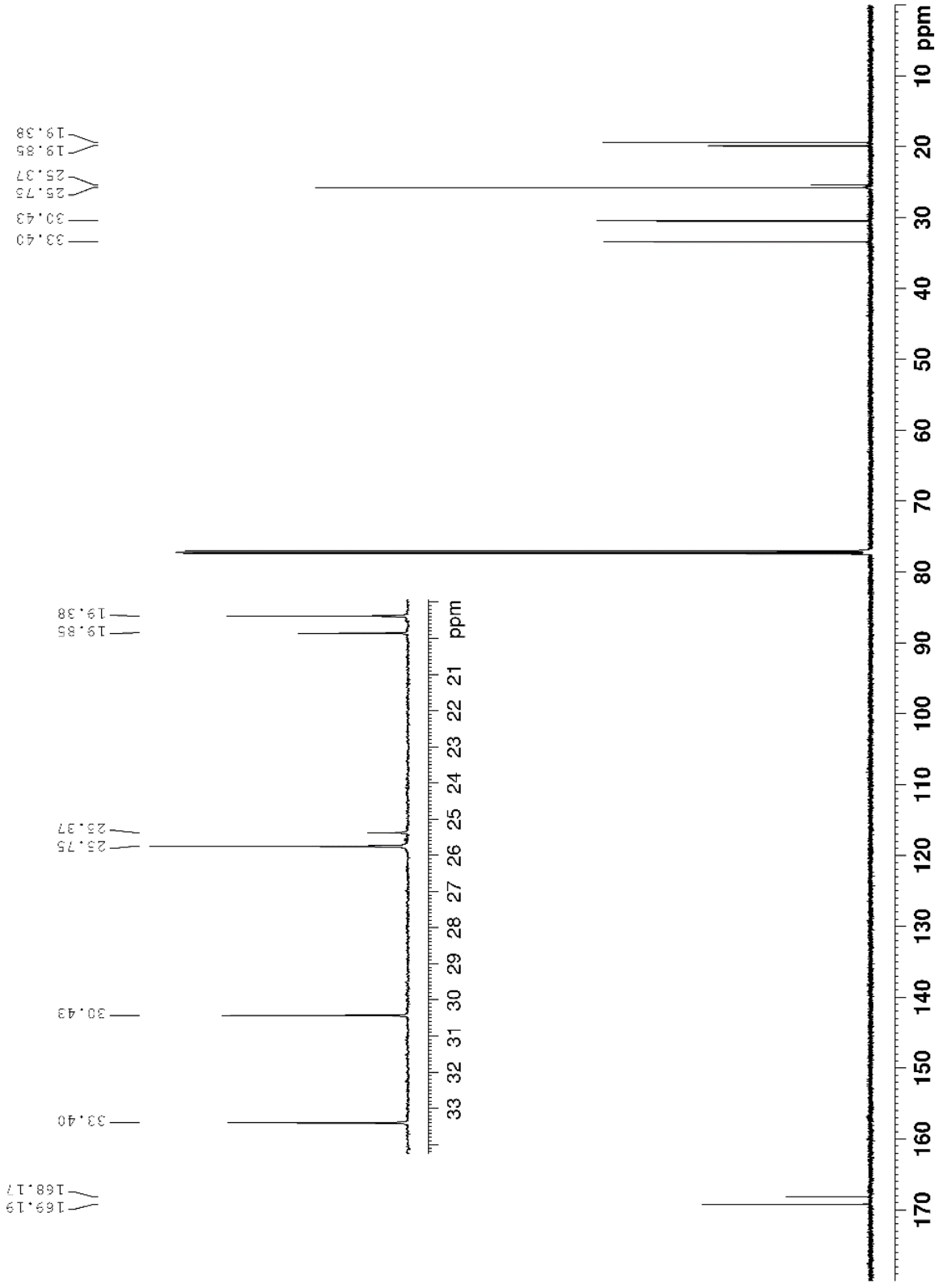


Figure 20: $^{13}C\{^{1}H\}$ NMR of 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate synthesized in reactionware.

4.4 Synthesis of Sodium 1-{[3-(3-methyl-3H-diazirin-3yl)propanoyl] oxy} 2,5-dioxopyrrolidine-3-sulfonate

To reduce the risk of any light induced decomposition throughout the synthesis, the modules were covered with aluminium foil whenever practical.

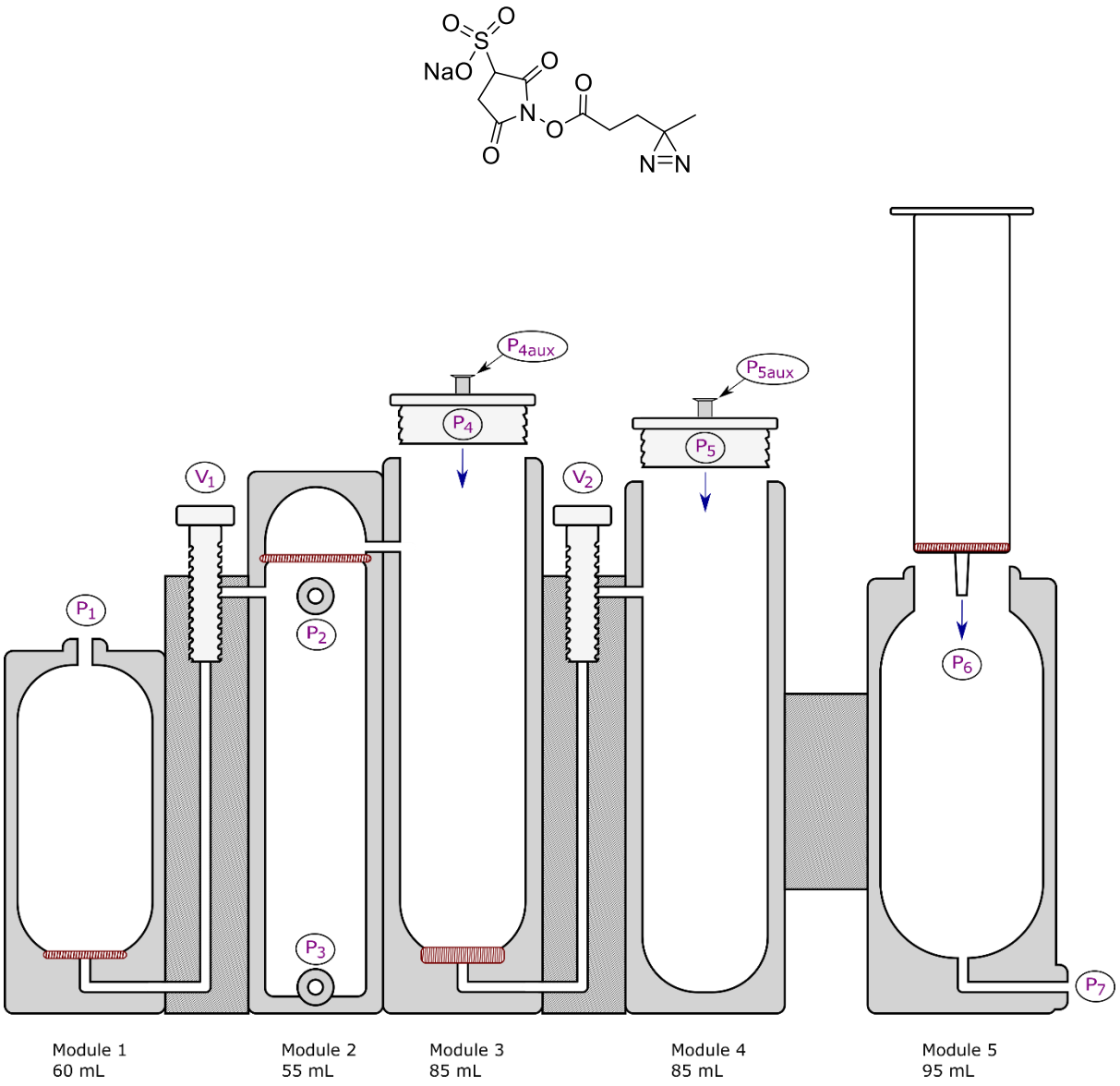


Figure 21: Schematic representation of cross section of the monolithic reactor used in syntheses of Sulfo-NHS-diazirines

4.4.1 Synthetic procedure in reactionware

Levulinic acid (0.60 g, 5.167 mmol) dissolved in 1.5 mL of anhydrous methanol was added into the module 1, through port P_1. The reaction vessel was placed in a cooling bath at 0℃. 7M solution of ammonia in methanol (9 ml, 63 mmol, 12.2 equiv) was then added into module 1 and the mixture stirred under nitrogen atmosphere for 1 h. The module was opened briefly to add 1.1 g of dried magnesium sulfate. The mixture was stirred for additional 5 h at 0℃. Hydroxylamine-O-sulfonic acid (0.701 g, 6.204 mmol, 1.2 equiv) was dissolved in 5.5 mL of anhydrous MeOH and added into the reaction module dropwise via syringe, over a course of 5 min. The reaction was then stirred for additional 16 h at 0℃. The cooling was stopped and the reaction mixture transferred to module 2 by applying gentle inert gas pressure to port P_1. The solvent was then evaporated by means of vacuum connected to port P_2. Note that evaporation was carried out at 20℃ to avoid decomposition and at vacuum not exceeding 50 mbar to avoid loss of the intermediate via evaporation. Following removal of the solvent and ammonia, module 2 was flushed with argon and the crude of diaziridine re-dissolved in 7 mL of anhydrous MeOH introduced via port P_2. The reaction monolith was placed inside an ice bath again and N,N-dimethylethylamine (0.57 mL, 5.26 mmol, 1.02 equiv) was introduced, through port P_2. This was stirred for 3 min, after which gradual addition of iodine beads commenced. When adding the iodine, colour of the reaction mixture became brown initially, but within a few seconds the colour disappeared to give a colourless solution again. Upon further addition of iodine, the rate of the disappearance of the brown colour slowed significantly. When the brown colour faded within 10 min of the last addition giving yellow tinted solution at the end of the 10 min period, the addition of iodine was stopped. Note that 0.811 g, 3.195 mmol, 0.618 equiv of iodine was normally required. The reaction was then left to stir for another 30 min. The ice bath was then removed and 3 mL of aqueous KI solution (50% w/v) was introduced into the module, through port P_2. This was followed by 1.2 mL of 50% w/v aqueous solution of ascorbic acid and finally 1 mL of 3M HCl, added dropwise. The mixture was extracted immediately with 70 ml of diethyl ether introduced via port P_3. Finally, 35 mL of saturated solution of sodium chloride was also introduced to enhance the recovery of the organic phase from the reaction module. The remaining contents of module 2 were then withdrawn via port P_3 and discarded. Note that upon addition of the extraction solvent and the sodium chloride solution to module 2, the organic phase containing the product

flowed into module 3 preloaded with vacuum dried (at 120℃) $MgSO_4$. The extract was dried overnight at ambient temperature. The following day, the organic extract was transferred to module 4 by means of gentle inert gas pressure applied to port P_{4aux} of module 3. Once transferred, vacuum was applied to port P_{5aux} in order to evaporate the solvent. Note that care must be taken during evaporation of the solvent as high vacuum applied for extended periods of time will cause loss of yield due to evaporation of the product itself. For this reason, evaporation was generally carried out at 20-25℃ and vacuum not exceeding 50 mbar. Once completed, the vacuum supply was cut off and module 4 was filled with argon. Port P_5 was then opened briefly and 10 mL of anhydrous DMF was added in via a syringe. Care was taken to wash down any crude that may have deposited on the walls of the module during evaporation. The crude was stirred briefly and N,N'-dicyclohexylmethanediimine (0.637 g, 3.087 mmol) was added into the solution as solid. Port P_5 was closed and the mixture stirred for 10 min. The module was then re-opened and sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate (0.558 g, 2.572 mmol) was added in as solid. Port P_5 was closed again. The gas from module 4 was evacuated via port P_{5aux} and replaced with fresh portion of argon. The monolith was placed in a water bath and the reaction mixture stirred for 40 h at 25℃. The water bath was then replaced with sodium chloride ice bath and the reaction stirred for 4 h. Port P_5 was opened to proceed with a filtration step. To begin a moderate negative pressure was applied to port P_7, while a gentle flow of dry nitrogen gas was applied to the top opening of the filter attachment (TELOS Phase Separator, 25ml), connected to the top of module 5 at port P_6. Glass pipette was used to quickly transfer the cold suspension from module 4 into the filter attachment. 2.5 mL of freezer chilled, anhydrous DMF was added into module 4 to recover any reminders of the crude from the module. Additional 2.5 mL of the ice cold, anhydrous DMF was used to rinse the precipitate caught by the filter. Negative pressure was closed off at port P_7 and the flow of nitrogen gas was stopped. The filter attachment was detached from port P_6 and discarded along with the trapped precipitate of dicyclohexylurea and residual sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate. 80 mL of anhydrous ethyl acetate was then added through the port into the filtrate contained in module 5. Port P_6 was closed with a rubber septum. The resultant mixture was stirred briefly and left at 2℃ for 20 h in order to precipitate the product. Note that a lower precipitation temperature can result in lower purity product without noticeable increase in yield. The white precipitate of the product was

withdrawn through port 8 and filtered through grade 5 Whatman filter paper (pore size 2.5 μm), under continuous flow of dry nitrogen gas. Fresh anhydrous ethyl acetate (10 mL) was then added into module 5 to recover any remaining precipitate in the form of a suspension, which was also filtered. 12 mL of fresh anhydrous ethyl acetate was then used directly to rinse the product on the filter, followed by 12 mL of anhydrous diethyl ether and 5 mL of anhydrous pentane. The filter paper along with the white precipitate was then dried overnight in a desiccator under vacuum. The dried product weighed 0.581 g, 1.775 mmol, giving yield 34% and purity 95%. ^{1}H NMR (600.1 MHz; 303 K; D_2O; δ, ppm; *J*, Hz): 4.54 (1H; bs), 3.44 (1H; bs), 3.24 (1H; dd; 3.1, 18.9), 2.75 (2H; t; 7.4), 1.88 (2H; t; 7.4), 1.11 (3H; s); $^{13}C\{^{1}H\}$ NMR (150.9 MHz, 303 K, D_2O; δ, ppm): 172.9, 172.3, 169.0, 59.4, 32.8, 31.4, 28.7, 28.2, 21.3.

Note that upon dissolution in D_2O the product begins to decompose, hence it is crucial to prepare NMR sample immediately before carrying out NMR experiment. We found that D_2O, despite this drawback, was superior in comparison with DMSO-d_6 since better peak separation could be achieved in both $^{13}C\{^{1}H\}$ and ^{1}H spectra.

4.4.2 Design and optimization of the cartridge geometry

Initially, each step in the synthetic process requiring a designated module was carried out in such a module as a discrete unit. This strategy was adopted to ensure that each module performed as required and intermediate outcomes in the synthesis were of satisfactory quality. Once optimal design parameters were resolved, the modules were being gradually connected together and tested for their performance as part of an assembly. Following this approach the entire system of modules was developed and optimised to best fulfil its purpose.

Since the entire synthetic process was enclosed in monolithic system formed out of five modules it was necessary to ensure that no undesired transfer of a reaction mixture was possible between neighbouring units. To ensure this, screw valves were fitted to the siphons located between the vessels. The valves were normally closed, until it became necessary to transfer reaction content from one unit to another. Semitransparency of the polypropylene modules generally allowed to gauge a level of liquid and its colour inside a module. Whenever

this was not sufficient, the required area of a module was backlit with a low power LED torch (Ansmann T50F, 60 lumens).

It is worth noting that heat transfer through polypropylene walls is poor when compared to heat transfer through glass walls of traditional glassware. With this in mind, during precipitation of dicyclohexylurea and residual sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate, lower temperature was employed to ensure that these compounds were stopped by filtration and could not act as an impurity at later stage. 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate was of particular concern, as it is readily soluble in DMF and often small amounts of it contaminated the product, unless sufficient cooling was employed in the proceeding step.

4.4.3 Operations sequence

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
1	0/00:00 +00:01	Add levulinic acid (0.6 g) dissolved in 1.5 mL of anhydrous methanol, to module 1, through port P_1	O	C	C	C	C	C	C	C	C	C
2	0/00:01 +00:01	Place the cartridge in a cooling bath at 0°C	O	C	C	C	C	C	C	C	C	C
3	0/00:02 +00:04	Add 9 mL of 7M ammonia in methanol, through port P_1	O	C	C	C	C	C	C	C	C	C
4	0/00:06 +00:01	Connect a nitrogen filled balloon to P_1	O N_2	C	C	C	C	C	C	C	C	C
5	0/00:07 +01:00	Stir for 1 h	O N_2	C	C	C	C	C	C	C	C	C
6	0/01:07 +00:01	Disconnect the balloon from P_1	O	C	C	C	C	C	C	C	C	C
7	0/01:08 +00:02	Add 1.1 g of dried $MgSO_4$, through port P_1	O	C	C	C	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
8	0/01:10 +00:01	Connect nitrogen filled balloon to P_1	O N_2	C	C	C	C	C	C	C	C	C
9	0/01:11 +05:00	Stir for 5 h at 0°C	O N_2	C	C	C	C	C	C	C	C	C
10	0/06:11 +00:01	Disconnect the balloon from P_1	O	C	C	C	C	C	C	C	C	C
11	0/06:12 +00:05	Add dropwise a solution of hydroxylamine-O-sulfonic acid (0.701 g) dissolved in 5.5 mL of dry methanol, via a syringe connected to port P_1	O	C	C	C	C	C	C	C	C	C
12	0/06:17 +00:01	Close port P_1	C	C	C	C	C	C	C	C	C	C
13	0/06:18 +16:00	Stir for 16 h at 0°C	C	C	C	C	C	C	C	C	C	C
14	0/22:18 +00:01	Remove the cooling	C	C	C	C	C	C	C	C	C	C
15	0/22:19 +00:01	Open port P_1	O	C	C	C	C	C	C	C	C	C
16	0/22:20 +00:02	Open valve V_1 to enable transfer of liquid from module 1 to module 2	O	O	C	C	C	C	C	C	C	C
17	0/22:22 +00:01	Open port P_2	O	O	O	C	C	C	C	C	C	C
18	0/22:23 +00:00	Stir at 250 rpm	O	O	O	C	C	C	C	C	C	C
19	0/22:23 +00:10	Apply gentle inert gas pressure to port P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C	C
20	0/22:33 +00:01	Add 2 mL of dry methanol to module 1, through P_1	O	O	O	C	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
21	0/22:34 +00:05	Apply gentle inert gas pressure to P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C	C
22	0/22:39 +00:01	Add another 2 mL of dry methanol to module 1, through P_1	O	O	O	C	C	C	C	C	C	C
23	0/22:40 +00:05	Apply gentle inert gas pressure to P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C	C
24	0/22:45 +00:01	Close P_1	C	O	O	C	C	C	C	C	C	C
25	0/22:46 +00:02	Close valve V_1	C	C	O	C	C	C	C	C	C	C
26	0/22:48 +06:00	Apply vacuum [note 1] to P_2 to partially evaporate the solvent in module 2. Reduce the volume of the liquid to approximately 2 mL	C	C	O Vac	C	C	C	C	C	C	C
27	1/04:48 +00:02	Close the vacuum and connect a source of argon to port P_2. Re-equilibrate the pressure inside module 2. Leave connected to the argon source	C	C	O Arg	C	C	C	C	C	C	C
28	1/05:00 +00:01	Place the monolith in a cooling bath at 0°C	C	C	O Arg	C	C	C	C	C	C	C
29	1/05:01 +00:05	Wait 5 min	C	C	O Arg	C	C	C	C	C	C	C
30	1/05:06 +00:01	Disconnect the argon source	C	C	O	C	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
31	1/05:07 +00:01	Add 7 mL of dry methanol through P_2	C	C	O	C	C	C	C	C	C	C
32	1/05:08 +00:01	Stir for 1 min	C	C	O	C	C	C	C	C	C	C
33	1/05:09 +00:01	Add 0.57 mL of N,N-dimethylethylamine through port P_2	C	C	O	C	C	C	C	C	C	C
34	1/05:10 +00:03	Stir for 3 min	C	C	O	C	C	C	C	C	C	C
35	1/05:13 +02:00	Add I_2 in small portions as solid through port P_2[note 2] Maintain stirring at approximately 300 rpm	C	C	O	C	C	C	C	C	C	C
36	1/07:13 +00:30	Stir for additional 30 min after the last addition of I_2	C	C	C	C	C	C	C	C	C	C
37	1/07:43 +00:01	Remove the cooling	C	C	C	C	C	C	C	C	C	C
38	1/07:44 +00:01	Stir at 250 rpm and add 3 mL of aqueous KI solution (50 % w/v), through P_2.	C	C	O	C	C	C	C	C	C	C
39	1/07:45 +00:01	Add 1.2 mL of 50 % w/v aqueous solution of ascorbic acid, through P_2.	C	C	O	C	C	C	C	C	C	C
40	1/07:46 +00:02	Increase the rate of stirring to approximately 400 rpm. Use port P_2 to add 1 mL of 3M HCl, dropwise over a course of 2 min.	C	C	O	C	C	C	C	C	C	C
41	1/07:48 +00:01	Close port P_2	C	C	C	C	C	C	C	C	C	C
42	1/07:49 +00:00	Stop stirring	C	C	C	C	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
43	1/07:49 +00:03	Open port P_4 and add 3 g dried magnesium sulfate to module 3	C	C	C	C	O	C	C	C	C	C
44	1/07:52 +00:03	Close P_4 and open P_4aux	C	C	C	C	O Aux	C	C	C	C	C
45	1/07:55 +00:02	Introduce 20 mL of diethyl ether through valve at port P_3	C	C	C	O Solv	O Aux	C	C	C	C	C
46	1/07:57 +00:00	Stir for 30 sec at 350 rpm	C	C	C	O Solv	O Aux	C	C	C	C	C
47	1/07:57 +00:01	Stop stirring	C	C	C	O Solv	O Aux	C	C	C	C	C
48	1/07:58 +00:02	Introduce 10 mL of diethyl ether through valve at port P_3	C	C	C	O Solv	O Aux	C	C	C	C	C
49	1/08:00 +00:00	Stir for 30 sec at 350 rpm	C	C	C	O Solv	O Aux	C	C	C	C	C
50	1/08:00 +00:01	Stop stirring	C	C	C	O Solv	O Aux	C	C	C	C	C
51	1/08:01 +00:12	Repeat operations 48 – 50 four more times	C	C	C	O Solv	O Aux	C	C	C	C	C
52	1/08:13 +00:05	Ensure the stirring if off. Slowly introduce 35 mL of saturated aqueous sodium chloride solution through port P_3	C	C	C	O Sol	O Aux	C	C	C	C	C
53	1/08:18 +00:03	Withdraw the waste liquid from module 2 through P_3	C	C	C	O	O Aux	C	C	C	C	C
54	1/08:21 +00:01	Close ports P_3 and P_4aux	C	C	C	C	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
55	1/08:22 +16:00	Place the cartridge in a dark place at ambient temperature, to allow for drying of the organic extract	C	C	C	C	C	C	C	C	C	C
56	2/00:22 +00:03	Open ports P_4aux and P_5aux. Open valve V_2	C	C	C	C	O Aux	O	O Aux	C	C	C
57	2/00:25 +00:05	Apply gentle inert gas pressure to P_4aux and transfer the liquid from module 3 to module 4	C	C	C	C	O Aux	O	O Aux	C	C	C
58	2/00:30 +00:02	Open port P_4 and add 5 mL of dry diethyl ether into module 3 note 3	C	C	C	C	O	O	O Aux	C	C	C
59	2/00:32 +00:02	Close P_4	C	C	C	C	O Aux	O	O Aux	C	C	C
60	2/00:34 +00:03	Apply gentle inert gas pressure to P_4aux and transfer the liquid from module 3 to module 4	C	C	C	C	O Aux	O	O Aux	C	C	C
61	2/00:37 +00:14	Repeat operations 58 – 60 two more times	C	C	C	C	O/ Aux	O	O Aux	C	C	C
62	2/00:51 +00:03	Close port P_4aux and valve V_2	C	C	C	C	C	C	O Aux	C	C	C
63	2/00:53 +00:00	Start stirring at 250 rpm	C	C	C	C	C	C	O Aux	C	C	C
64	2/00:53 +06:00	Apply vacuum to port P_5aux and evaporate the solvent from module 4 note 4	C	C	C	C	C	C	O Aux -Vac	C	C	C
65	2/06:53 +00:02	Once completed, close the vacuum and fill module 4 with argon	C	C	C	C	C	C	O Aux -Arg	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
66	2/06:55 +00:02	Open port P_5 and add 10 mL of anh DMF to module 4[note 5]	C	C	C	C	C	C	O	C	C	C
67	2/06:57 +00:01	Close port P_5	C	C	C	C	C	C	C	C	C	C
68	2/06:58 +00:03	Stir for 3 min	C	C	C	C	C	C	C	C	C	C
69	2/07:01 +00:01	Open port P_5	C	C	C	C	C	C	O	C	C	C
70	2/07:02 +00:01	Add N,N'-dicyclohexylmethane-diimine (0.637 g) as solid	C	C	C	C	C	C	O	C	C	C
71	2/07:03 +00:01	Close port P_5	C	C	C	C	C	C	C	C	C	C
72	2/07:04 +00:10	Stir for 10 min	C	C	C	C	C	C	C	C	C	C
73	2/07:14 +00:01	Open port P_5	C	C	C	C	C	C	O	C	C	C
74	2/07:15 +00:01	Add sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate (0.558 g) as solid	C	C	C	C	C	C	O	C	C	C
75	2/07:16 +00:01	Close port P_5	C	C	C	C	C	C	C	C	C	C
76	2/07:17 +00:01	Open port P_5aux	C	C	C	C	C	C	O Aux	C	C	C
77	2/07:18 +00:02	Connect vacuum supply and evacuate gas from module 4	C	C	C	C	C	C	O Aux -Vac	C	C	C
78	2/07:20 +00:02	Close the vacuum and re-equilibrate pressure in module 4 by introducing argon gas through port P_5aux	C	C	C	C	C	C	O Aux -Arg	C	C	C
79	2/07:22 +00:02	Place the monolith in a water bath. Set the temperature to 25℃	C	C	C	C	C	C	O Aux -Arg	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
80	2/07:24 +1/16:00	Stir for 40 h	C	C	C	C	C	C	O Aux -Arg	C	C	C
81	3/23:24 +00:02	Replace the water bath with an ice bath	C	C	C	C	C	C	O Aux -Arg	C	C	C
82	3/23:26 +04:00	Stir for 4 h at -15 to -20°C note 6	C	C	C	C	C	C	O Aux -Arg	C	C	C
83	4/03:26 +00:02	Open port P_6 and connect the filter attachment	C	C	C	C	C	C	O Aux -Arg	O	C	C
84	4/03:28 +00:02	Open port P_7 and apply a moderate negative pressure to the port	C	C	C	C	C	C	O Aux -Arg	O	O	C
85	4/03:30 +00:02	Apply a gentle flow of dry nitrogen gas to the top opening of the filter attachment, connected to port P_6	C	C	C	C	C	C	O Aux -Arg	O N_2	O	C
86	4/03:32 +00:01	Open port P_5	C	C	C	C	C	C	O	O N_2	O	C
87	4/03:33 +00:02	Use a glass pipette to quickly transfer the cold suspension from module 4 into the filter attachment	C	C	C	C	C	C	O	O N_2	O	C
88	4/03:35 +00:02	Add 2.5 mL of freezer chilled anh DMF to module 4 and recover any remining precipitate as suspension	C	C	C	C	C	C	O	O N_2	O	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
89	4/03:37 +00:01	Wash precipitate stopped by the filter with another 2.5 mL of freezer chilled anh DMF	C	C	C	C	C	C	O	O N_2	O	C
89	4/03:38 +00:01	Stop negative pressure at port P_7	C	C	C	C	C	C	O	O N_2	O	C
90	4/03:39 +00:01	Stop the flow of nitrogen gas at the top opening of the filter attachment, connected to port P_6	C	C	C	C	C	C	O	O	O	C
91	4/03:40 +00:00	Detach filter attachment from port P_6	C	C	C	C	C	C	O	O	O	C
92	4/03:40 +00:01	Close port P_5	C	C	C	C	C	C	C	O	O	C
93	4/03:41 +00:01	Close port P_7	C	C	C	C	C	C	C	O	C	C
94	4/03:42 +00:05	Add 80 mL of anh ethyl acetate through port P_6	C	C	C	C	C	C	C	O	C	C
95	4/03:47 +00:01	Stir for 1 min	C	C	C	C	C	C	C	O	C	C
96	4/03:48 +00:00	Close port P_6 with a rubber septum	C	C	C	C	C	C	C	C	C	C
97	4/03:48 +20:00	Place the monolith at 2°C for 20 h	C	C	C	C	C	C	C	C	C	C
98	4/23:48 +00:01	Stop the cooling	C	C	C	C	C	C	C	C	C	C
99	4/23:49 +00:01	Open port P_6	C	C	C	C	C	C	C	O	C	C
100	4/23:50 +00:10	Withdraw the suspension from module 5 via port P_8 and filter outside of the of the monolith [note 7]	C	C	C	C	C	C	C	O	C	O

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
101	5/00:00 +00:05	Add 10 mL of anh ethyl acetate to module 5 via port P_6, to recover any remaining precipitate in form of suspension	C	C	C	C	C	C	C	O	C	O
102	5/00:05 +00:03	Rinse the precipitate with 12 mL of anh ethyl acetate	C	C	C	C	C	C	C	C	C	C
103	5/00:08 +00:03	Rinse the precipitate with 12 mL of anh diethyl ether	C	C	C	C	C	C	C	C	C	C
104	5/00:11 +00:02	Rinse the precipitate with 5 mL of anh pentane	C	C	C	C	C	C	C	C	C	C
105	5/00:13 +20:00	Dry overnight under vacuum	C	C	C	C	C	C	C	C	C	C
106	5/20:13	Store the product at -18°C, under argon, away from light.										

note 1: Note that evaporation was carried out at 20°C to avoid decomposition and at vacuum not exceeding 50 mbar to avoid loss of the intermediate via evaporation.

note 2: When adding the iodine, the colour of the reaction mixture becomes brown initially, but within a few seconds the colour disappears to give a colourless solution again. Upon further addition of iodine, the rate of the disappearance of the brown colour slows significantly. When the brown colour fades within 10 min of the last addition giving yellow tinted solution at the end of the 10 min period, the addition of iodine should be stopped.

note 3: Use syringe. Use the solvent to wash down any crude that may have deposited on the walls of the module.

note 4: initially use gentle vacuum (~900 mbar) to avoid a boil-off of the solvent and loss of the organic extract into the vacuum line. Over time gradually increase the power of the vacuum. Note that care must be taken during evaporation of the solvent as high vacuum applied for extended periods of time will cause loss of yield due to evaporation of the product itself. For this reason, evaporation was generally carried out at 20-25°C and vacuum not exceeding 50 mbar.

note 5: Take care to wash down any crude that may have deposited on the walls of the module during evaporation.

note 6: Ensure the cooling remains efficient throughout the prescribed time.

note 7: Filter through grade 5 Whatman filter paper (pore size 2.5 μm), under continuous flow of dry nitrogen gas.

4.4.4 NMR analysis of product synthesized in reactionware.

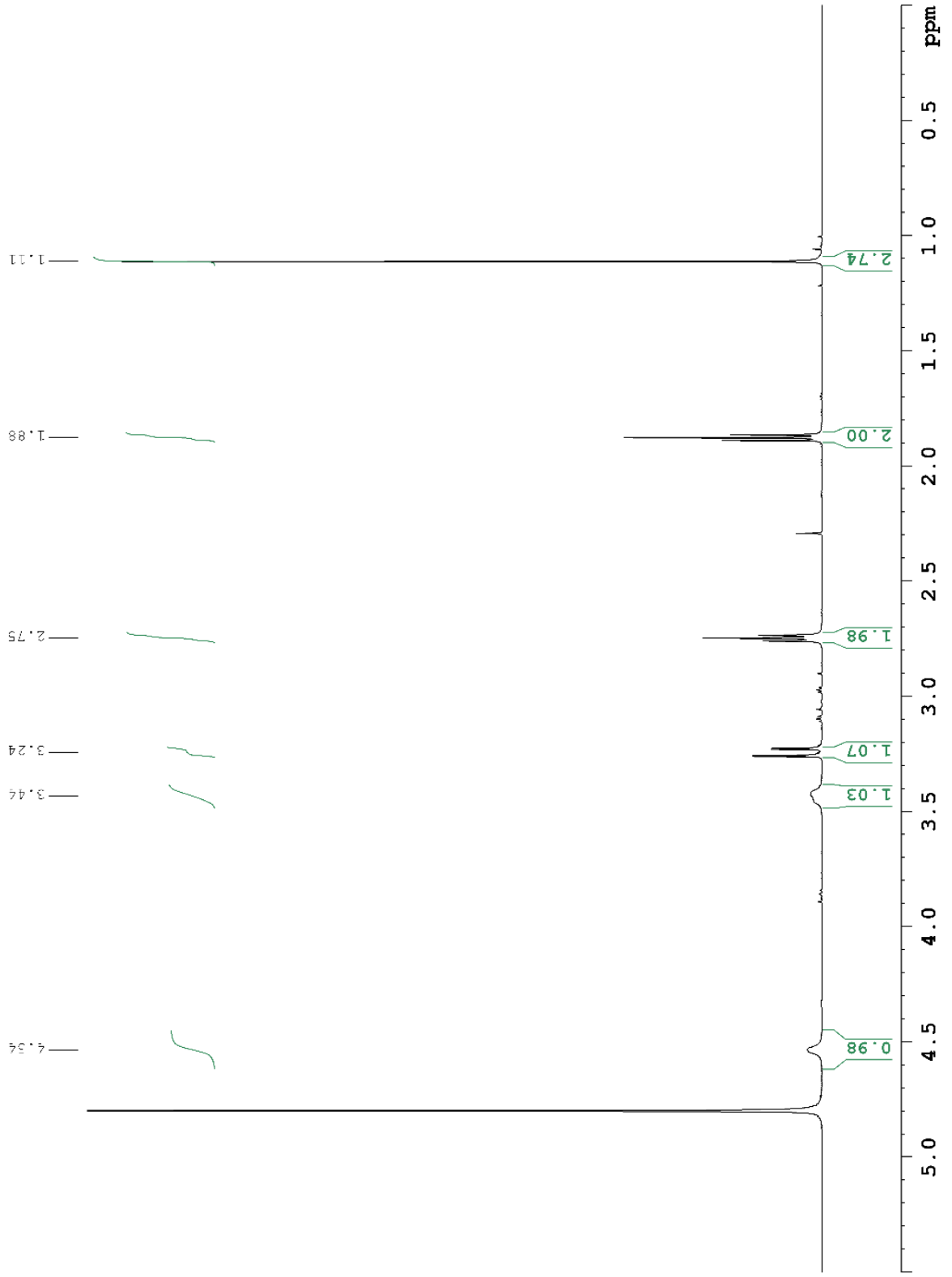


Figure 22: ^{1}H NMR of Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy} 2,5-dioxopyrrolidine-3-sulfonate.

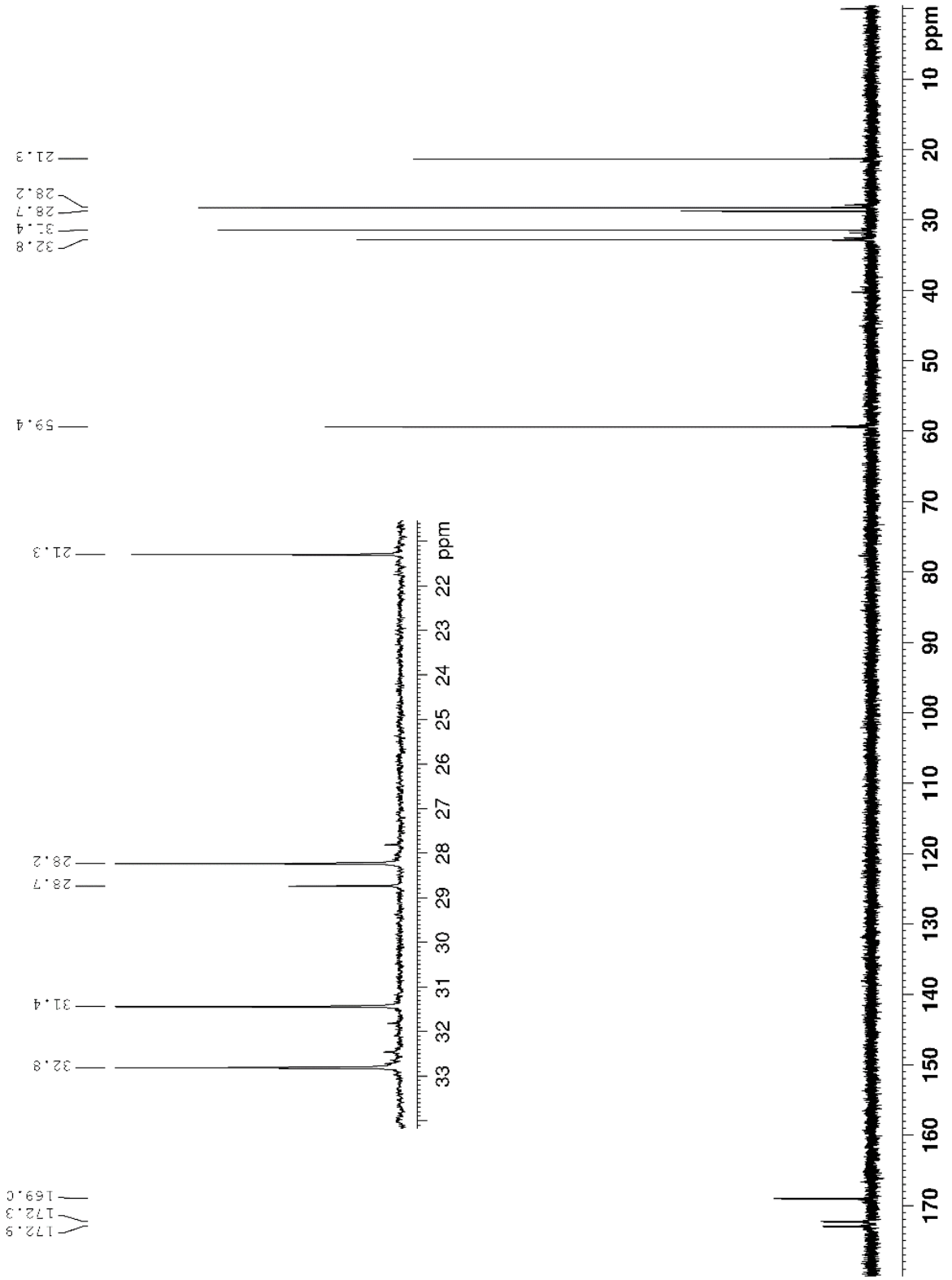


Figure 23: $^{13}C\{^{1}H\}$ NMR of Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate.

4.5 Synthesis of Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl) butanoyl] oxy}-2,5-dioxopyrrolidine-3 sulfonate

To reduce the risk of any light induced decomposition throughout the synthesis, the modules were covered with aluminium foil whenever practical.

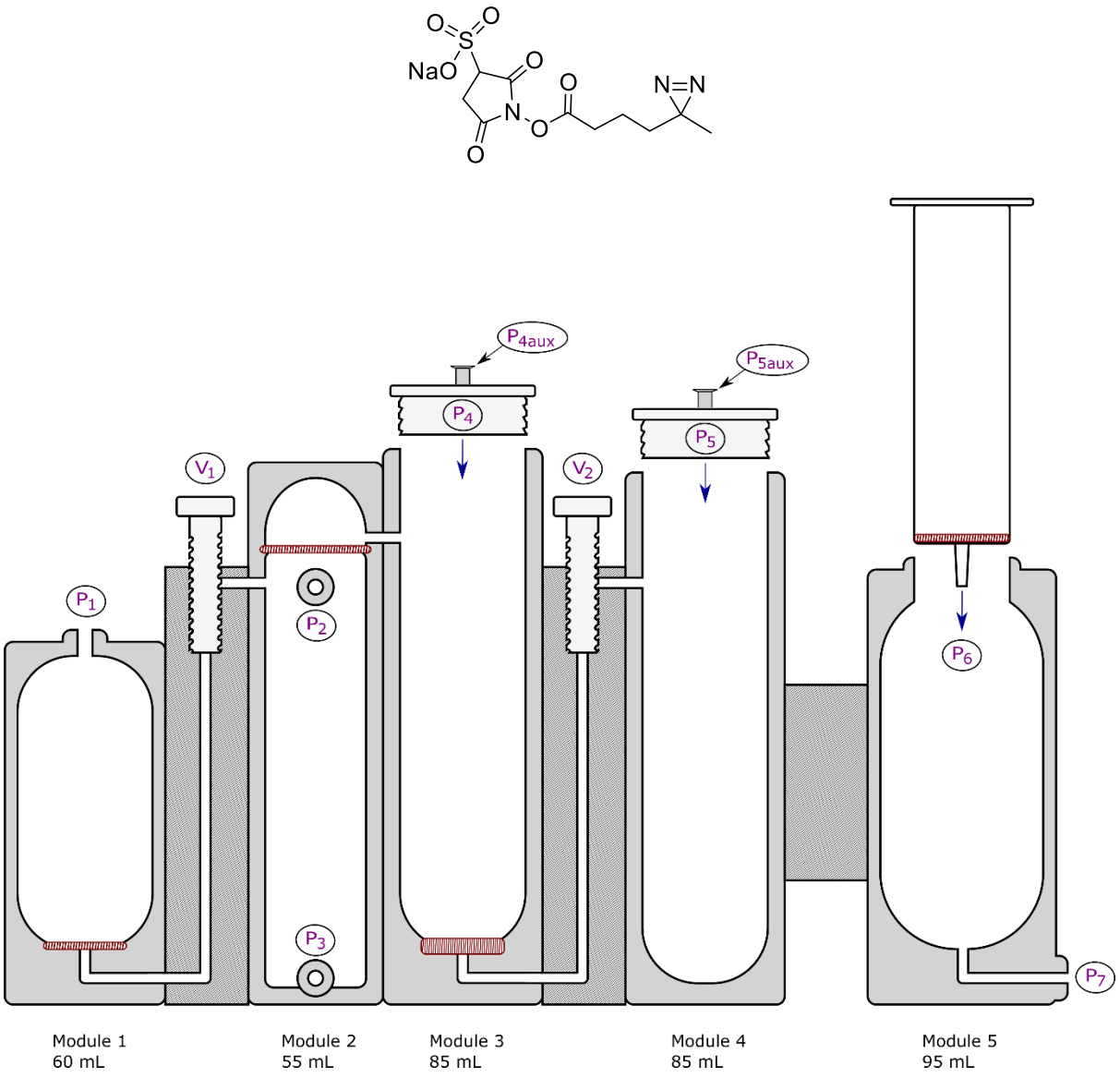


Figure 24: Schematic representation of cross section of the monolithic reactor used in syntheses of Sulfo-NHS-diazirines

4.5.1 Synthetic procedure in reactionware

4-Acetylbutyric acid (0.673 g, 5.17 mmol) dissolved in 1.5 mL of anhydrous methanol was added into module 1, through port P_1. The reaction vessel was placed in a cooling bath at 0°C. 7M solution of ammonia in methanol (9 ml, 63 mmol, 12.2 equiv) was then added into module 1 and the mixture stirred under nitrogen atmosphere for 1 h. The module was opened briefly to add 1.1 g of dried magnesium sulfate. The mixture was stirred for additional 5 h at 0°C. Hydroxylamine-O-sulfonic acid (0.701 g, 6.204 mmol, 1.2 equiv) was dissolved in 5.5 mL of anhydrous MeOH and added into the reaction module dropwise via syringe, over a course of 5 min. The reaction was then stirred for additional 16 h at 0°C. The cooling was stopped and the reaction mixture transferred to module 2 by applying gentle inert gas pressure to port P_1. The solvent was then evaporated by means of vacuum connected to port P_2. Module 2 was then flushed with argon and the crude of diaziridine re-dissolved in 7 mL of anhydrous MeOH introduced via port P_2. The reaction monolith was placed inside an ice bath again and N,N-dimethylethylamine (0.57 mL, 5.26 mmol, 1.02 equiv) was introduced, through port P_2. This was stirred for 5 min, after which gradual addition of iodine beads commenced. When adding the iodine, colour of the reaction mixture became brown initially, but within a few seconds the colour disappeared to give a colourless solution again. Upon further addition of iodine, the rate of the disappearance of the brown colour slowed significantly. When the brown colour faded within 10 min of the last addition giving yellow tinted solution at the end of the 10 min period, the addition of iodine was stopped. Note that 0.743 g, 2.927 mmol, 0.57 equiv of iodine was normally required. The reaction was then left to stir for another 30 min. The ice bath was then removed and 3 mL of aqueous KI solution (50% w/v) was introduced into the module, through port P_2. This was followed by 1.2 mL of 50% w/v aqueous solution of ascorbic acid and finally 1 mL of 3M HCl, added dropwise. The mixture was extracted immediately with 70 ml of diethyl ether introduced via port P_3. Finally, 35 mL of saturated solution of sodium chloride was also introduced to enhance the recovery of the organic phase from the reaction module. The remaining contents of module 2 were then withdrawn via port P_3 and discarded. Note that upon addition of the extraction solvent and the sodium chloride solution to module 2, the organic phase containing the product flowed into module 3 preloaded with vacuum dried (at 120°C) $MgSO_4$. The extract was dried overnight at ambient temperature. The following day, the organic extract was transferred to module 4 by means of

gentle inert gas pressure applied to port P_{4aux} of module 3. Once transferred, vacuum was applied to port P_{5aux} in order to evaporate the solvent. Once completed, the vacuum supply was cut off and module 4 was filled with argon. Port P_5 was then opened briefly and 10 mL of anhydrous DMF was added in via a syringe. Care was taken to wash down any crude that may have deposited on the walls of the module during evaporation. The crude was stirred briefly and N,N'-dicyclohexylmethanediimine (0.576 g, 2.792 mmol) was added into the solution as solid. Port P_5 was closed and the mixture stirred for 10 min. The module was then re-opened and sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate (0.505 g, 2.326 mmol) was added in as solid. Port P_5 was closed again. The gas from module 4 was evacuated via port P_{5aux} and replaced with fresh portion of argon. The monolith was placed in a water bath and the reaction mixture stirred for 40 h at 25℃. The water bath was then replaced with sodium chloride ice bath and the reaction stirred for 4 h. Port P_5 was opened to proceed with a filtration step. To begin a moderate negative pressure was applied to port P_7, while a gentle flow of dry nitrogen gas was applied to the top opening of the filter attachment (TELOS Phase Separator, 25ml), connected to the top of module 5 at port P_6. Glass pipette was used to quickly transfer the cold suspension from module 4 into the filter attachment. 2.5 mL of freezer chilled, anhydrous DMF was added into module 4 to recover any reminders of the crude from the module. Additional 2.5 mL of the ice cold, anhydrous DMF was used to rinse the precipitate caught by the filter. Negative pressure was closed off at port P_7 and the flow of nitrogen gas stopped. The filter attachment was detached from port P_6 and discarded along with the trapped precipitate of dicyclohexylurea and residual sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate. 80 mL of anhydrous ethyl acetate was then added through the port into the filtrate contained in module 5. Port P_6 was closed with a rubber septum. The resultant mixture was stirred briefly and left at 2℃ for 20 h in order to precipitate the product. Note that a lower precipitation temperature can result in lower purity product without noticeable increase in yield. The white precipitate of the product was withdrawn through port 8 and filtered through grade 5 Whatman filter paper (pore size 2.5 μm), under continuous flow of dry nitrogen gas. Fresh anhydrous ethyl acetate (10 mL) was then added into module 5 to recover any remaining precipitate in the form of a suspension, which was also filtered. 12 mL of fresh anhydrous ethyl acetate was then used directly to rinse the product on the filter, followed by 12 mL of anhydrous diethyl ether and 5 mL of anhydrous pentane. The filter

paper along with the white precipitate was then dried overnight in a desiccator under vacuum. The dry product weighed 0.517 g, 1.515 mmol, giving yield 29% and purity 93%. ^{1}H NMR (600.1 MHz; 303 K; D_2O; δ, ppm; *J*, Hz): 4.53 (1H; bs), 3.43 (1H; bs), 3.24 (1H; dd; 3.1, 18.9), 2.79 (2H; t; 7.3), 1.71 (2H; m), 1.52 (2H; t; 7.8), 1.08 (3H; s); ^{13}C{^{1}H} NMR (150.9 MHz, 303 K, D_2O; δ, ppm): 173.0, 172.7, 169.1, 59.4, 35.4, 32.8, 32.6, 29.3, 21.5, 21.4

Note that upon dissolution in D_2O the product begins to decompose, hence it is crucial to prepare NMR sample immediately before carrying out NMR experiment. We found that D_2O, despite this drawback, was superior in comparison with DMSO-d_6 since better peak separation could be achieved in both ^{13}C{^{1}H} and ^{1}H spectra.

4.5.2 Design and optimization of the cartridge geometry

Initially, each step in the synthetic process requiring a designated module was carried out in such a module as a discrete unit. This strategy was adopted to ensure that each module performed as required and intermediate outcomes in the synthesis were of satisfactory quality. Once optimal design parameters were resolved, the modules were being gradually connected together and tested for their performance as part of an assembly. Following this approach, the entire system of modules was developed and optimised to best fulfil its purpose.

Since the entire synthetic process was enclosed in monolithic system formed out of five modules it was necessary to ensure that no undesired transfer of a reaction mixture was possible between neighbouring units. To ensure this, screw valves were fitted to the siphons located between the vessels. The valves were normally closed, until it became necessary to transfer reaction content from one unit to another. Semitransparency of the polypropylene modules generally allowed to gauge a level of liquid and its colour inside a module. Whenever this was not sufficient, the required area of a module was backlit with a low power LED torch (Ansmann T50F, 60 lumens).

It is worth noting that heat transfer through polypropylene walls is poor when compared to heat transfer through glass walls of traditional glassware. With this in mind, during precipitation of dicyclohexylurea and residual sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate, lower temperature was employed to ensure that these compounds were stopped

by filtration and could not act as an impurity at later stage. 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate was of particular concern, as it is readily soluble in DMF and often small amounts of it contaminated the product, unless sufficient cooling was employed in the proceeding step.

4.5.3 Operations sequence

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
1	0/00:00 +00:01	Add 4-Acetylbutyric acid (0.673 g) dissolved in 1.5 mL of anhydrous methanol, to module 1, through port P_1	O	C	C	C	C	C	C	C	C	C
2	0/00:01 +00:01	Place the cartridge in a cooling bath at 0°C	O	C	C	C	C	C	C	C	C	C
3	0/00:02 +00:04	Add 9 mL of 7M ammonia in methanol, through port P_1	O	C	C	C	C	C	C	C	C	C
4	0/00:06 +00:01	Connect a nitrogen filled balloon to P_1	O N_2	C	C	C	C	C	C	C	C	C
5	0/00:07 +01:00	Stir for 1 h	O N_2	C	C	C	C	C	C	C	C	C
6	0/01:07 +00:01	Disconnect the balloon from P_1	O	C	C	C	C	C	C	C	C	C
7	0/01:08 +00:02	Add 1.1 g of dried $MgSO_4$, through port P_1	O	C	C	C	C	C	C	C	C	C
8	0/01:10 +00:01	Connect nitrogen filled balloon to P_1	O N_2	C	C	C	C	C	C	C	C	C
9	0/01:11 +05:00	Stir for 5 h at 0°C	O N_2	C	C	C	C	C	C	C	C	C
10	0/06:11 +00:01	Disconnect the balloon from P_1	O	C	C	C	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
11	0/06:12 +00:05	Add dropwise a solution of hydroxylamine-O-sulfonic acid (0.701 g) dissolved in 5.5 mL of dry methanol, via a syringe connected to port P_1	O	C	C	C	C	C	C	C	C	C
12	0/06:17 +00:01	Close port P_1	C	C	C	C	C	C	C	C	C	C
13	0/06:18 +16:00	Stir for 16 h at 0°C	C	C	C	C	C	C	C	C	C	C
14	0/22:18 +00:01	Remove the cooling	C	C	C	C	C	C	C	C	C	C
15	0/22:19 +00:01	Open port P_1	O	C	C	C	C	C	C	C	C	C
16	0/22:20 +00:02	Open valve V_1 to enable transfer of liquid from module 1 to module 2	O	O	C	C	C	C	C	C	C	C
17	0/22:22 +00:01	Open port P_2	O	O	O	C	C	C	C	C	C	C
18	0/22:23 +00:00	Stir at 250 rpm	O	O	O	C	C	C	C	C	C	C
19	0/22:23 +00:10	Apply gentle inert gas pressure to port P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C	C
20	0/22:33 +00:01	Add 2 mL of dry methanol to module 1, through P_1	O	O	O	C	C	C	C	C	C	C
21	0/22:34 +00:05	Apply gentle inert gas pressure to P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C	C
22	0/22:39 +00:01	Add another 2 mL of dry methanol to module 1, through P_1	O	O	O	C	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
23	0/22:40 +00:05	Apply gentle inert gas pressure to P_1 and transfer the solution from module 1 to module 2	O	O	O	C	C	C	C	C	C	C
24	0/22:45 +00:01	Close P_1	C	O	O	C	C	C	C	C	C	C
25	0/22:46 +00:02	Close valve V_1	C	C	O	C	C	C	C	C	C	C
26	0/22:48 +06:00	Apply vacuum [note 1] to P_2 to partially evaporate the solvent in module 2. Reduce the volume of the liquid to approximately 2 mL	C	C	O Vac	C	C	C	C	C	C	C
27	1/04:48 +00:02	Close the vacuum and connect a source of argon to port P_2. Re-equilibrate the pressure inside module 2. Leave connected to the argon source	C	C	O Arg	C	C	C	C	C	C	C
28	1/05:00 +00:01	Place the monolith in a cooling bath at 0°C	C	C	O Arg	C	C	C	C	C	C	C
29	1/05:01 +00:05	Wait 5 min	C	C	O Arg	C	C	C	C	C	C	C
30	1/05:06 +00:01	Disconnect the argon source	C	C	O	C	C	C	C	C	C	C
31	1/05:07 +00:01	Add 7 mL of dry methanol through P_2	C	C	O	C	C	C	C	C	C	C
32	1/05:08 +00:01	Stir for 1 min	C	C	O	C	C	C	C	C	C	C
33	1/05:09 +00:01	Add 0.57 mL of N,N-dimethylethylamine through port P_2	C	C	O	C	C	C	C	C	C	C
34	1/05:10 +00:03	Stir for 3 min	C	C	O	C	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
35	1/05:13 +02:00	Add I_2 in small portions as solid through port P_2 note 2 Maintain stirring at approximately 300 rpm	C	C	O	C	C	C	C	C	C	C
36	1/07:13 +00:30	Stir for additional 30 min after the last addition of I_2	C	C	C	C	C	C	C	C	C	C
37	1/07:43 +00:01	Remove the cooling	C	C	C	C	C	C	C	C	C	C
38	1/07:44 +00:01	Stir at 250 rpm and add 3 mL of aqueous KI solution (50 % w/v), through P_2.	C	C	O	C	C	C	C	C	C	C
39	1/07:45 +00:01	Add 1.2 mL of 50 % w/v aqueous solution of ascorbic acid, through P_2.	C	C	O	C	C	C	C	C	C	C
40	1/07:46 +00:02	Increase the rate of stirring to approximately 400 rpm. Use port P_2 to add 1 mL of 3M HCl, dropwise over a course of 2 min.	C	C	O	C	C	C	C	C	C	C
41	1/07:48 +00:01	Close port P_2	C	C	C	C	C	C	C	C	C	C
42	1/07:49 +00:00	Stop stirring	C	C	C	C	C	C	C	C	C	C
43	1/07:49 +00:03	Open port P_4 and add 3 g dried magnesium sulfate to module 3	C	C	C	C	O	C	C	C	C	C
44	1/07:52 +00:03	Close P_4 and open P_4aux	C	C	C	C	O Aux	C	C	C	C	C
45	1/07:55 +00:02	Introduce 20 mL of diethyl ether through valve at port P_3	C	C	C	O Solv	O Aux	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
46	1/07:57 +00:00	Stir for 30 sec at 350 rpm	C	C	C	O Solv	O Aux	C	C	C	C	C
47	1/07:57 +00:01	Stop stirring	C	C	C	O Solv	O Aux	C	C	C	C	C
48	1/07:58 +00:02	Introduce 10 mL of diethyl ether through valve at port P_3	C	C	C	O Solv	O Aux	C	C	C	C	C
49	1/08:00 +00:00	Stir for 30 sec at 350 rpm	C	C	C	O Solv	O Aux	C	C	C	C	C
50	1/08:00 +00:01	Stop stirring	C	C	C	O Solv	O Aux	C	C	C	C	C
51	1/08:01 +00:12	Repeat operations 48 – 50 four more times	C	C	C	O Solv	O Aux	C	C	C	C	C
52	1/08:13 +00:05	Ensure the stirring if off. Slowly introduce 35 mL of saturated aqueous sodium chloride solution through port P_3	C	C	C	O Sol	O Aux	C	C	C	C	C
53	1/08:18 +00:03	Withdraw the waste liquid from module 2 through P_3	C	C	C	O	O Aux	C	C	C	C	C
54	1/08:21 +00:01	Close ports P_3 and P_4aux	C	C	C	C	C	C	C	C	C	C
55	1/08:22 +16:00	Place the cartridge in a dark place at ambient temperature, to allow for drying of the organic extract	C	C	C	C	C	C	C	C	C	C
56	2/00:22 +00:03	Open ports P_4aux and P_5aux. Open valve V_2	C	C	C	C	O Aux	O	O Aux	C	C	C
57	2/00:25 +00:05	Apply gentle inert gas pressure to P_4aux and transfer the liquid from module 3 to module 4	C	C	C	C	O Aux	O	O Aux	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
58	2/00:30 +00:02	Open port P_4 and add 5 mL of dry diethyl ether into module 3 note 3	C	C	C	C	O	O	O Aux	C	C	C
59	2/00:32 +00:02	Close P_4	C	C	C	C	O Aux	O	O Aux	C	C	C
60	2/00:34 +00:03	Apply gentle inert gas pressure to P_4aux and transfer the liquid from module 3 to module 4	C	C	C	C	O Aux	O	O Aux	C	C	C
61	2/00:37 +00:14	Repeat operations 58 – 60 two more times	C	C	C	C	O/ Aux	O	O Aux	C	C	C
62	2/00:51 +00:03	Close port P_4aux and valve V_2	C	C	C	C	C	C	O Aux	C	C	C
63	2/00:53 +00:00	Start stirring at 250 rpm	C	C	C	C	C	C	O Aux	C	C	C
64	2/00:53 +06:00	Apply vacuum to port P_5aux and evaporate the solvent from module 4 note 4	C	C	C	C	C	C	O Aux -Vac	C	C	C
65	2/06:53 +00:02	Once completed, close the vacuum and fill module 4 with argon	C	C	C	C	C	C	O Aux -Arg	C	C	C
66	2/06:55 +00:02	Open port P_5 and add 10 mL of anh DMF to module 4 note 5	C	C	C	C	C	C	O	C	C	C
67	2/06:57 +00:01	Close port P_5	C	C	C	C	C	C	C	C	C	C
68	2/06:58 +00:03	Stir for 3 min	C	C	C	C	C	C	C	C	C	C
69	2/07:01 +00:01	Open port P_5	C	C	C	C	C	C	O	C	C	C
70	2/07:02 +00:01	Add N,N'-dicyclohexylmethane-diimine (0.576 g) as solid	C	C	C	C	C	C	O	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
71	2/07:03 +00:01	Close port P_5	C	C	C	C	C	C	C	C	C	C
72	2/07:04 +00:10	Stir for 10 min	C	C	C	C	C	C	C	C	C	C
73	2/07:14 +00:01	Open port P_5	C	C	C	C	C	C	O	C	C	C
74	2/07:15 +00:01	Add sodium 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate (0.505 g) as solid	C	C	C	C	C	C	O	C	C	C
75	2/07:16 +00:01	Close port P_5	C	C	C	C	C	C	C	C	C	C
76	2/07:17 +00:01	Open port P_5aux	C	C	C	C	C	C	O Aux	C	C	C
77	2/07:18 +00:02	Connect vacuum supply and evacuate gas from module 4	C	C	C	C	C	C	O Aux -Vac	C	C	C
78	2/07:20 +00:02	Close the vacuum and re-equilibrate pressure in module 4 by introducing argon gas through port P_5aux	C	C	C	C	C	C	O Aux -Arg	C	C	C
79	2/07:22 +00:02	Place the monolith in a water bath. Set the temperature to 25℃	C	C	C	C	C	C	O Aux -Arg	C	C	C
80	2/07:24 +1/16:00	Stir for 40 h	C	C	C	C	C	C	O Aux -Arg	C	C	C
81	3/23:24 +00:02	Replace the water bath with an ice bath	C	C	C	C	C	C	O Aux -Arg	C	C	C
82	3/23:26 +04:00	Stir for 4 h at -15 to -20℃ note 6	C	C	C	C	C	C	O Aux -Arg	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
83	4/03:26 +00:02	Open port P_6 and connect the filter attachment	C	C	C	C	C	C	O Aux -Arg	O	C	C
84	4/03:28 +00:02	Open port P_7 and apply a moderate negative pressure to the port	C	C	C	C	C	C	O Aux -Arg	O	O	C
85	4/03:30 +00:02	Apply a gentle flow of dry nitrogen gas to the top opening of the filter attachment, connected to port P_6	C	C	C	C	C	C	O Aux -Arg	O N_2	O	C
86	4/03:32 +00:01	Open port P_5	C	C	C	C	C	C	O	O N_2	O	C
87	4/03:33 +00:02	Use a glass pipette to quickly transfer the cold suspension from module 4 into the filter attachment	C	C	C	C	C	C	O	O N_2	O	C
88	4/03:35 +00:02	Add 2.5 mL of freezer chilled anh DMF to module 4 and recover any remining precipitate as suspension	C	C	C	C	C	C	O	O N_2	O	C
89	4/03:37 +00:01	Wash precipitate stopped by the filter with another 2.5 mL of freezer chilled anh DMF	C	C	C	C	C	C	O	O N_2	O	C
89	4/03:38 +00:01	Stop negative pressure at port P_7	C	C	C	C	C	C	O	O N_2	O	C
90	4/03:39 +00:01	Stop the flow of nitrogen gas at the top opening of the filter attachment, connected to port P_6	C	C	C	C	C	C	O	O	O	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
91	4/03:40 +00:00	Detach filter attachment from port P_6	C	C	C	C	C	C	O	O	O	C
92	4/03:40 +00:01	Close port P_5	C	C	C	C	C	C	C	O	O	C
93	4/03:41 +00:01	Close port P_7	C	C	C	C	C	C	C	O	C	C
94	4/03:42 +00:05	Add 80 mL of anh ethyl acetate through port P_6	C	C	C	C	C	C	C	O	C	C
95	4/03:47 +00:01	Stir for 1 min	C	C	C	C	C	C	C	O	C	C
96	4/03:48 +00:00	Close port P_6 with a rubber septum	C	C	C	C	C	C	C	C	C	C
97	4/03:48 +20:00	Place the monolith at 2°C for 20 h	C	C	C	C	C	C	C	C	C	C
98	4/23:48 +00:01	Stop the cooling	C	C	C	C	C	C	C	C	C	C
99	4/23:49 +00:01	Open port P_6	C	C	C	C	C	C	C	O	C	C
100	4/23:50 +00:10	Withdraw the suspension from module 5 via port P_8 and filter outside of the of the monolith note 7	C	C	C	C	C	C	C	O	C	O
101	5/00:00 +00:05	Add 10 mL of anh ethyl acetate to module 5 via port P_6, to recover any remaining precipitate in form of suspension	C	C	C	C	C	C	C	O	C	O
102	5/00:05 +00:03	Rinse the precipitate with 12 mL of anh ethyl acetate	C	C	C	C	C	C	C	C	C	C
103	5/00:08 +00:03	Rinse the precipitate with 12 mL of anh diethyl ether	C	C	C	C	C	C	C	C	C	C
104	5/00:11 +00:02	Rinse the precipitate with 5 mL of anh pentane	C	C	C	C	C	C	C	C	C	C

Op. No.	Time d/hh:mm	Action	P_1	V_1	P_2	P_3	P_4	V_2	P_5	P_6	P_7	P_8
105	5/00:13 +20:00	Dry overnight under vacuum	C	C	C	C	C	C	C	C	C	C
106	5/20:13	Store the product at -18°C, under argon, away from light.										

note 1: Note that evaporation was carried out at 20°C.

note 2: When adding the iodine, the colour of the reaction mixture becomes brown initially, but within a few seconds the colour disappears to give a colourless solution again. Upon further addition of iodine, the rate of the disappearance of the brown colour slows significantly. When the brown colour fades within 10 min of the last addition giving yellow tinted solution at the end of the 10 min period, the addition of iodine should be stopped.

note 3: Use syringe. Use the solvent to wash down any crude that may have deposited on the walls of the module.

note 4: initially use gentle vacuum (~900 mbar) to avoid a boil-off of the solvent and loss of the organic extract into the vacuum line. Over time gradually increase the power of the vacuum.

note 5: Take care to wash down any crude that may have deposited on the walls of the module during evaporation.

note 6: Ensure the cooling remains efficient throughout the prescribed time.

note 7: Filter through grade 5 Whatman filter paper (pore size 2.5 μm), under continuous flow of dry nitrogen gas.

4.5.4 NMR analysis of reactionware product

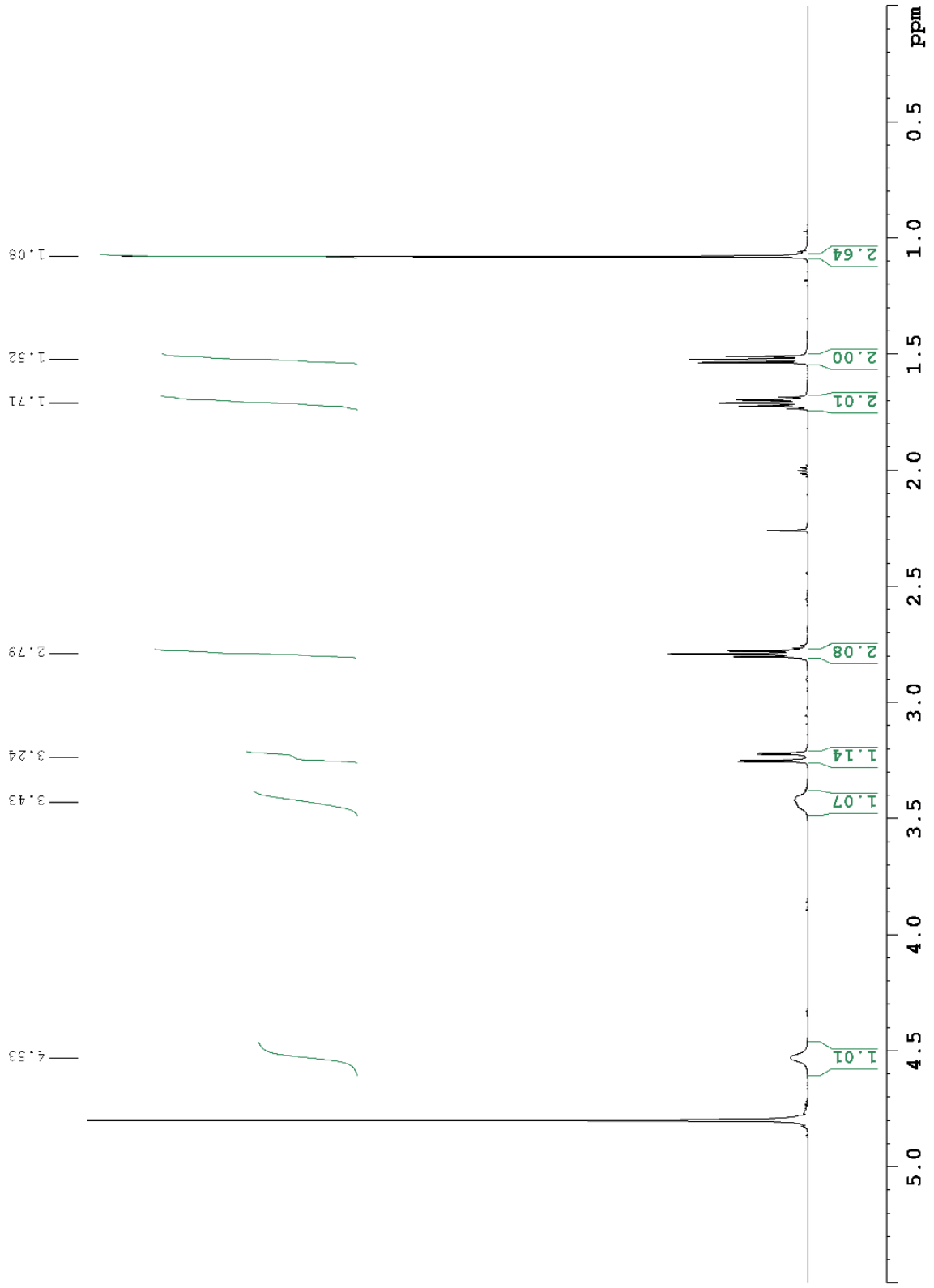


Figure 25: ^{1}H NMR of Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate synthesized in reactionware cartridge.

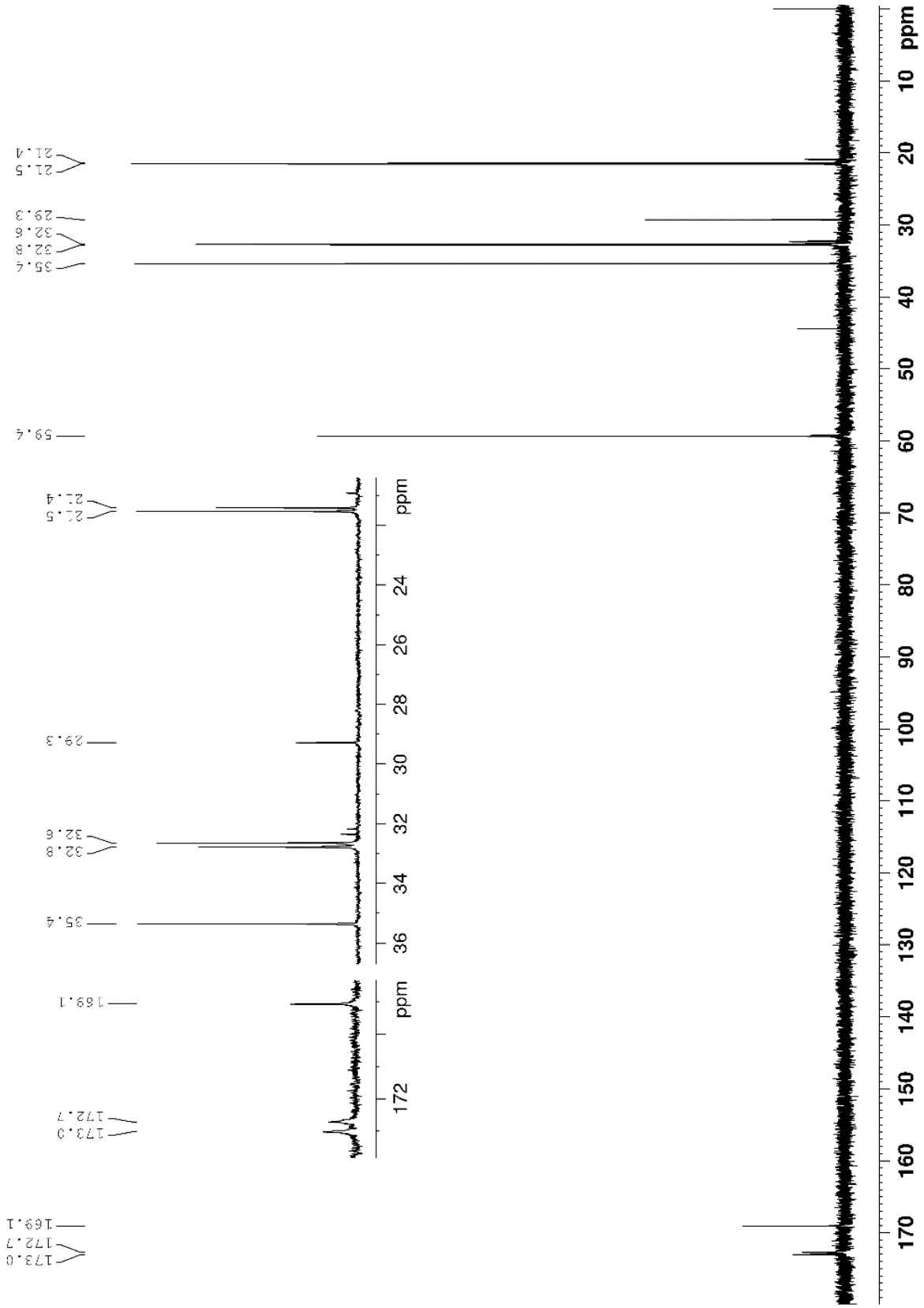


Figure 26: $^{13}C\{^{1}H\}$ NMR of Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate synthesized in reactionware cartridge.

4.6 Diazirine validation experiments

The substrate, Angiotensin II trifluoroacetate (5 mg) and the photoprobes (26.085 μmol) were dissolved in a mixture of 200 μL of deionized water and 300 μL of deuterated acetonitrile. The resultant mixtures were briefly vortexed and then left to stand at room temperature for a period of 30 min. The samples were then transferred into a wide mouth, 7 ml glass vial. The solutions were subsequently irradiated under UV light* for a period of 25 min. Following photoactivation, the sample was transferred into a NMR tube, additional 100 μL of 50:50 H_2O/acetonitrile mixture was used to rinse the vial and added to the total volume in the NMR tube. Subsequently DEPTQ NMR experiments were recorded to observe changes in the electronic structure of the peptide, as compared with a control experiment. The control experiment was carried out with peptide alone (no addition of diazirines) and irradiated by UV light, as described. Finally ESI mass spectroscopy experiments were also carried out to confirm existence of any predicted species, based on the observed masses.

*Note: The light source used for the photoactivation was a Xenon lamp (Oriel Instruments, Model 67005) with power output set to 100 Watts. The light was guided via 1 metre Liquid Light Guide (Newport). 355 nm bandpass filter was placed in the light path coming from the light guide and directed at the sample from a distance of approximately four centimetres.

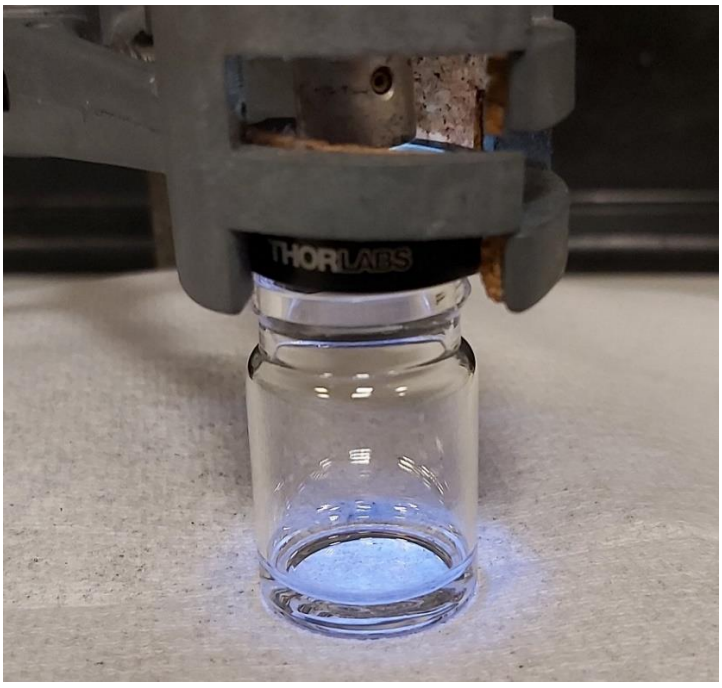


Figure 27: Photoactivation setup

4.6.1 Notes on the ESI-MS results

Note that based on the number of peaks present on the ESI spectra, we could conclude that a variety of products were formed as a result of interaction between our photo-probes and the substrate. The masses reported below were common to all of the experiments. The most commonly found peaks were those belonging to doubly charged species and hence represent half of the total masses of ionised molecules.

4.6.2 Mass spectroscopy results from validation experiments.

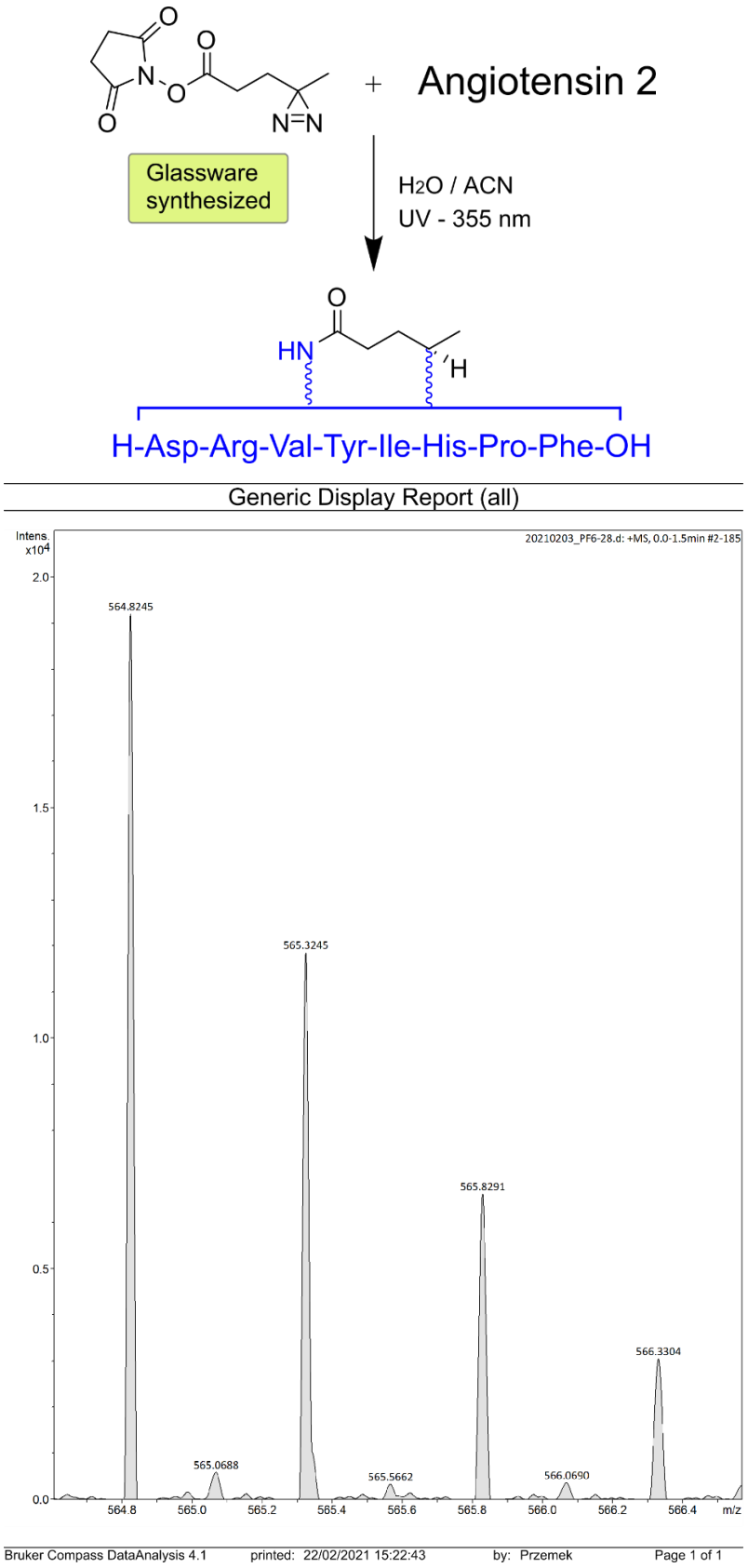


Figure 28: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate synthesized in glassware, and Angiotensin 2

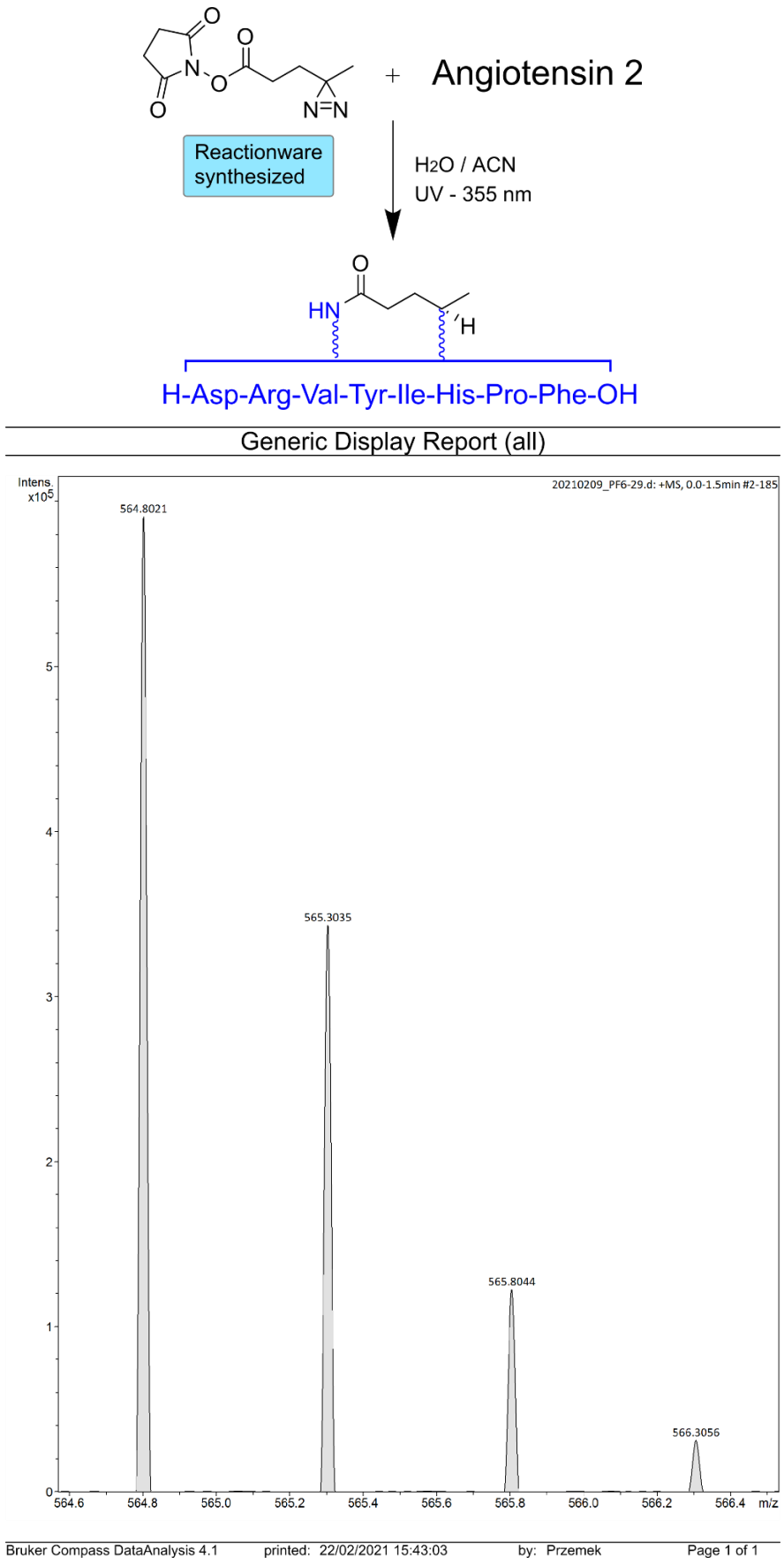


Figure 29: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate synthesized in reactionware, and Angiotensin 2

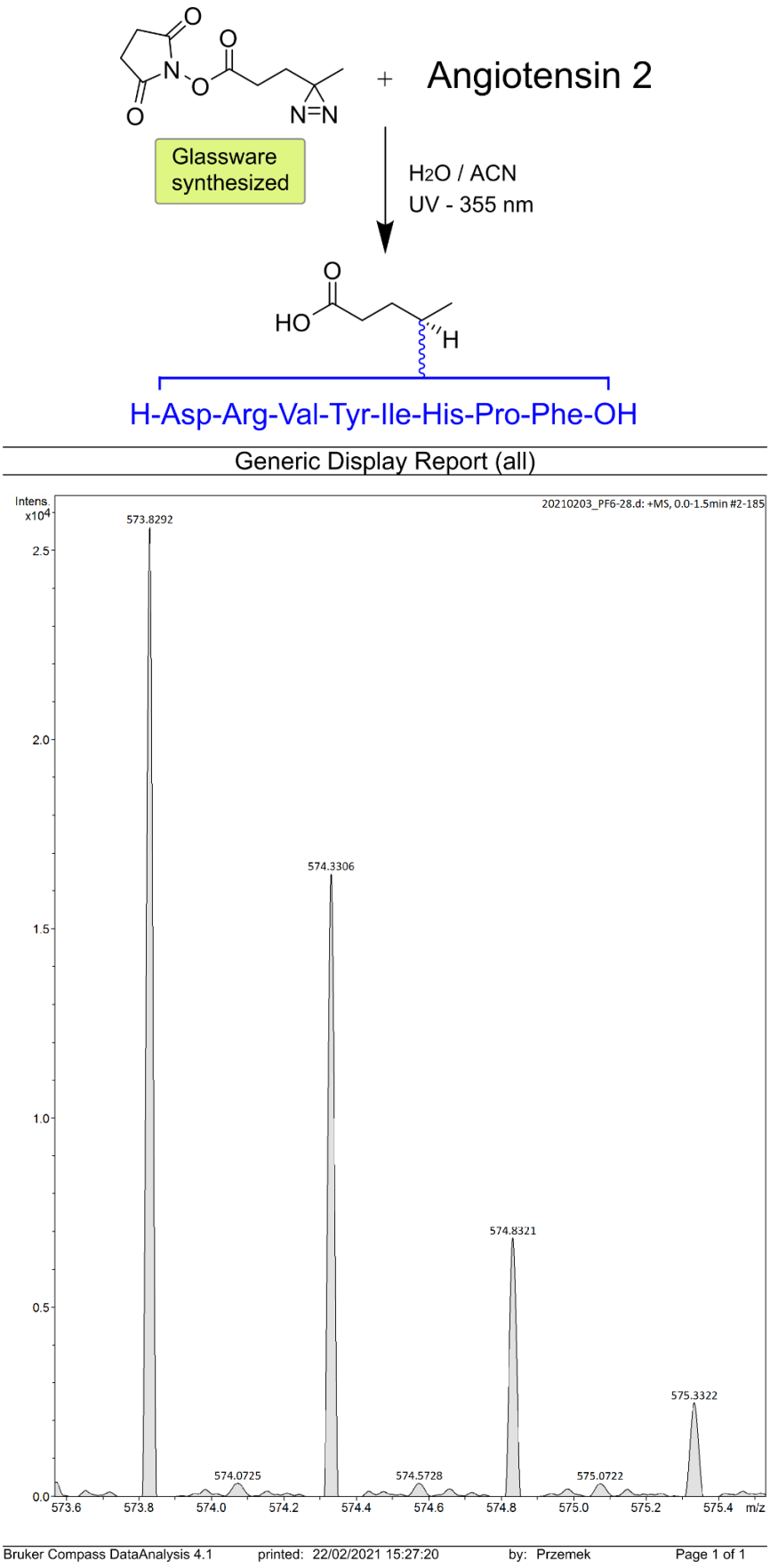


Figure 30: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate synthesized in glassware, and Angiotensin 2

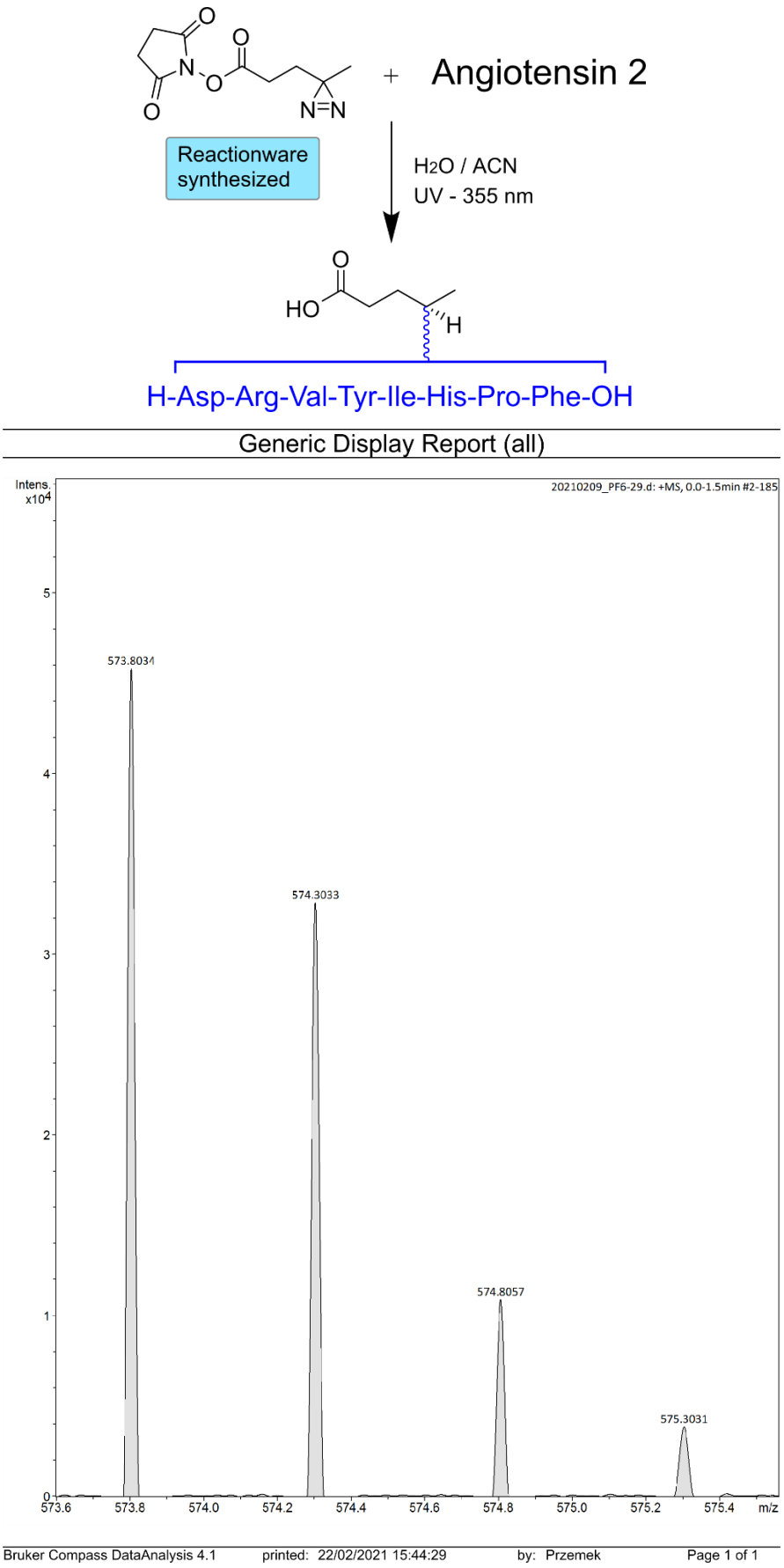


Figure 31: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate synthesized in reactionware, and Angiotensin 2

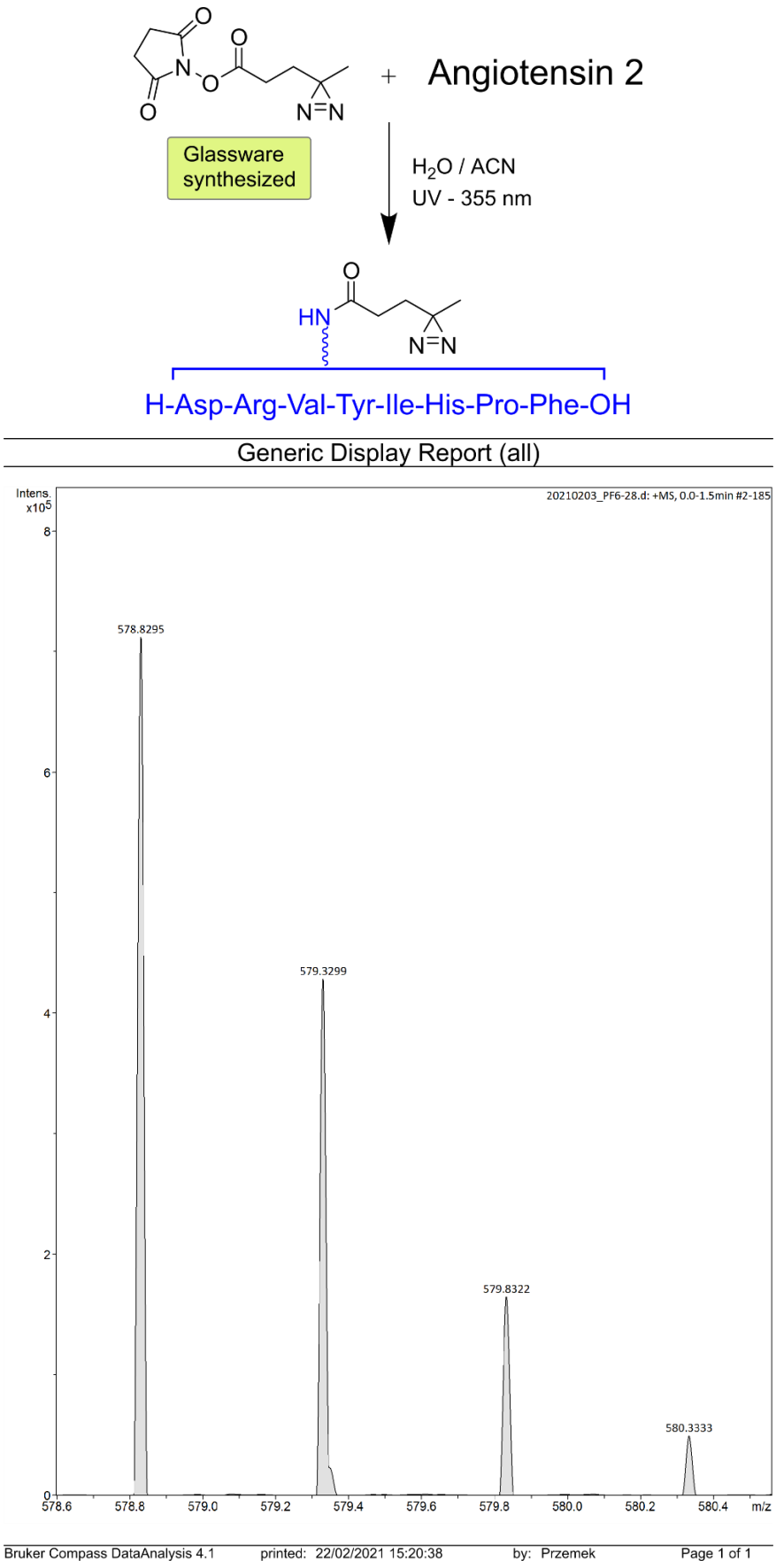


Figure 32: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate synthesized in glassware, and Angiotensin 2

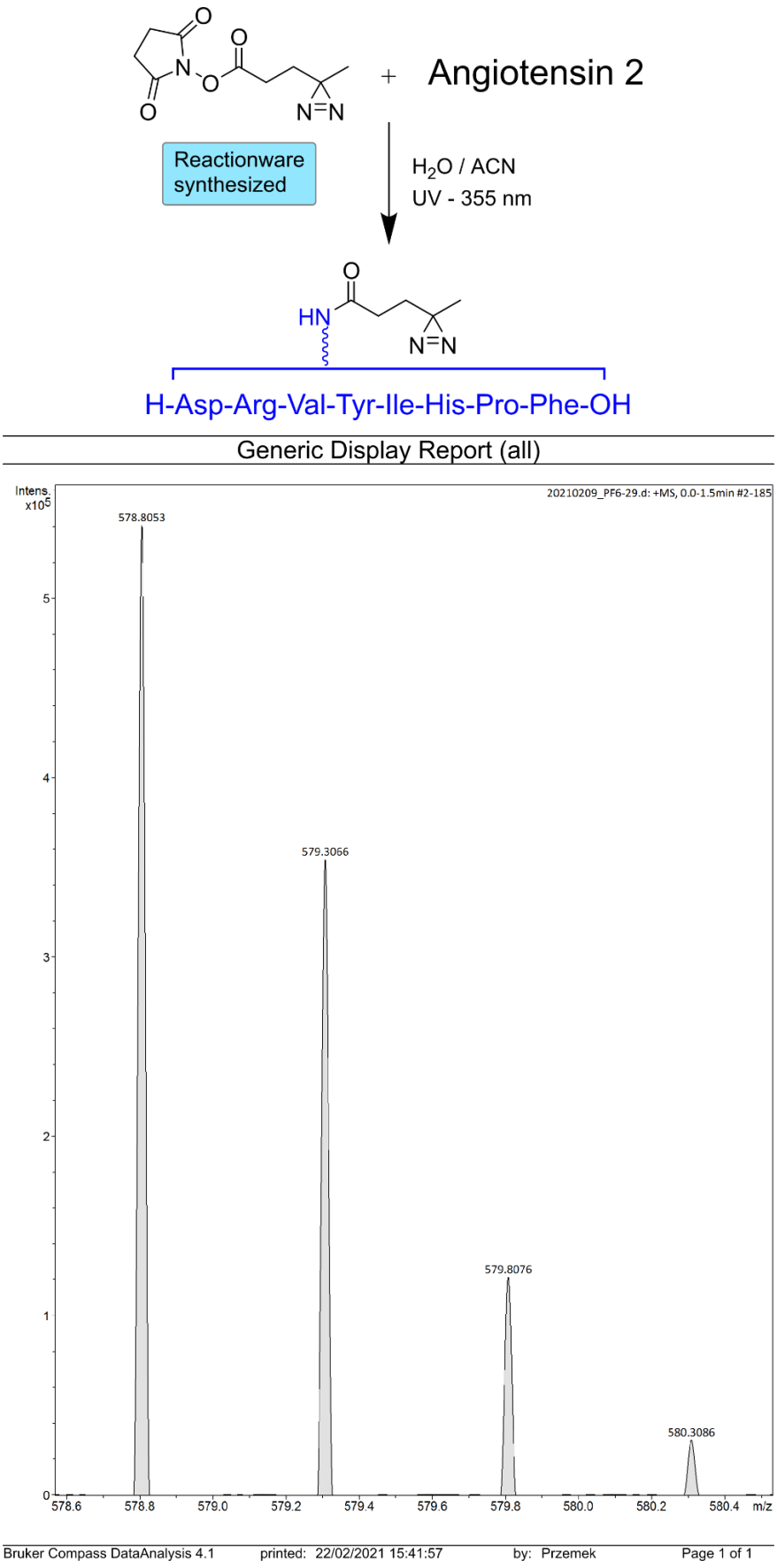


Figure 33: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate synthesized in reactionware, and Angiotensin 2

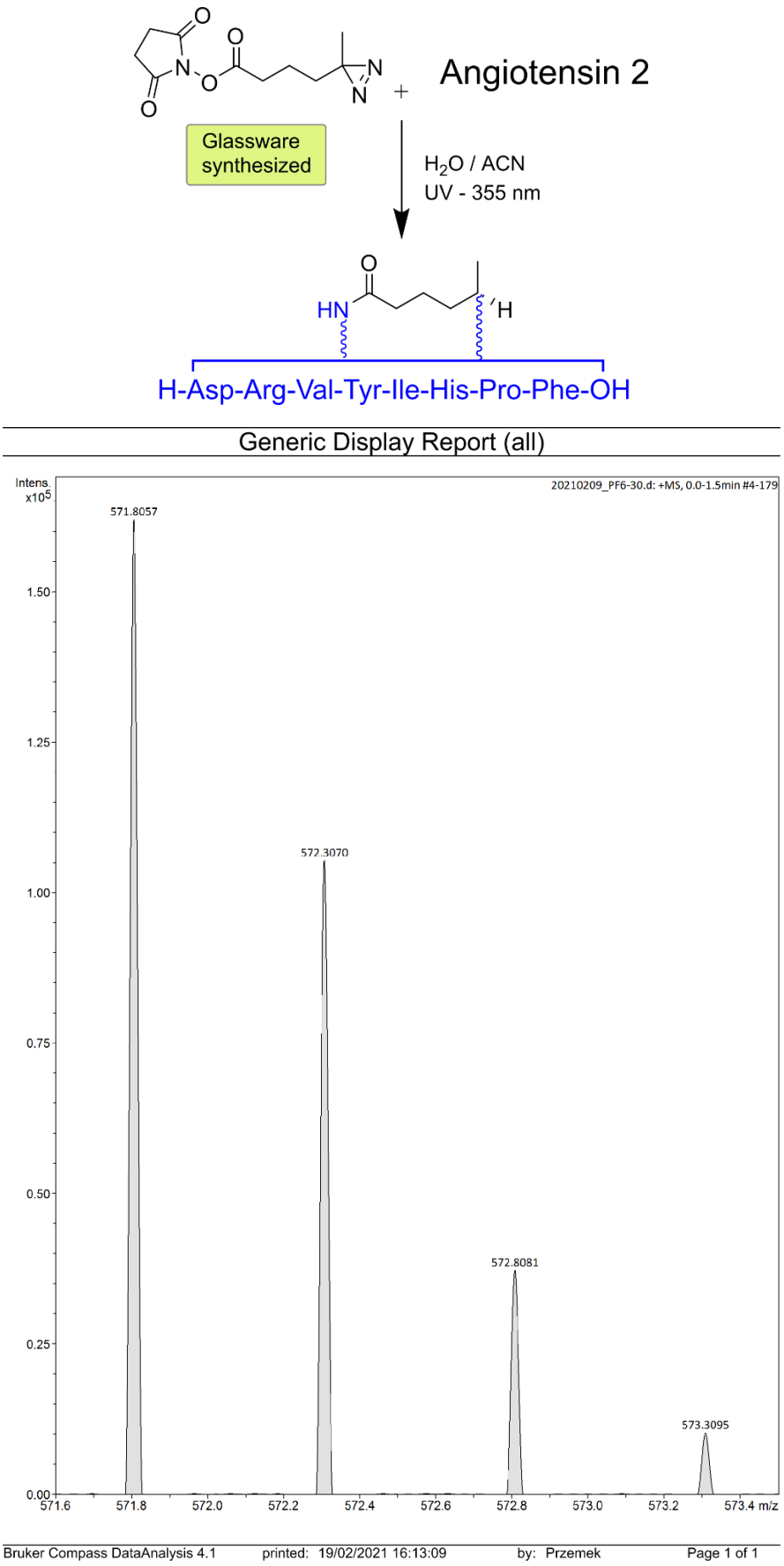


Figure 34: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate synthesized in glassware, and Angiotensin 2

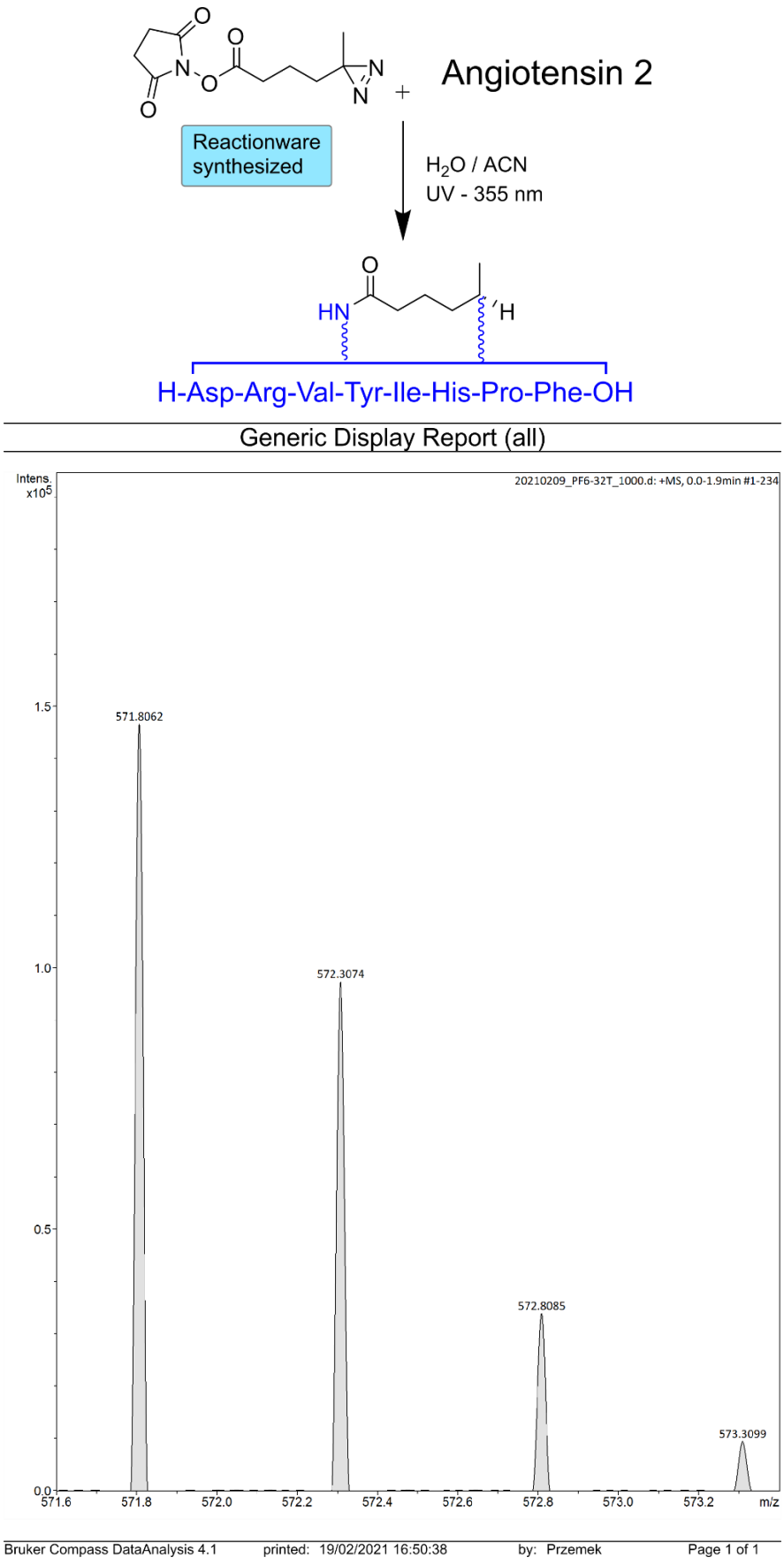
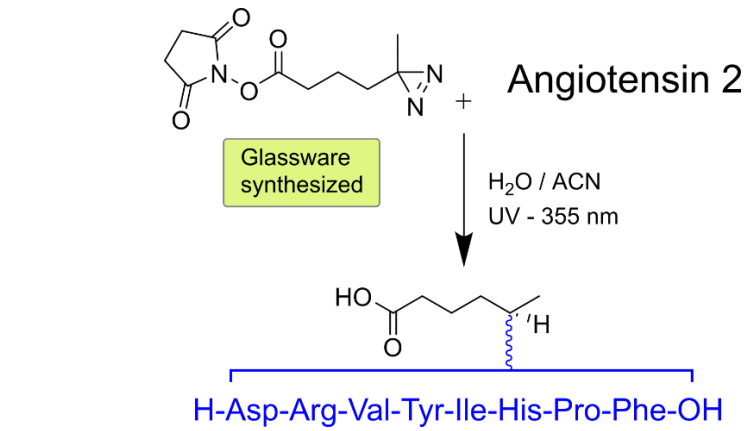


Figure 35: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate synthesized in reactionware, and Angiotensin 2



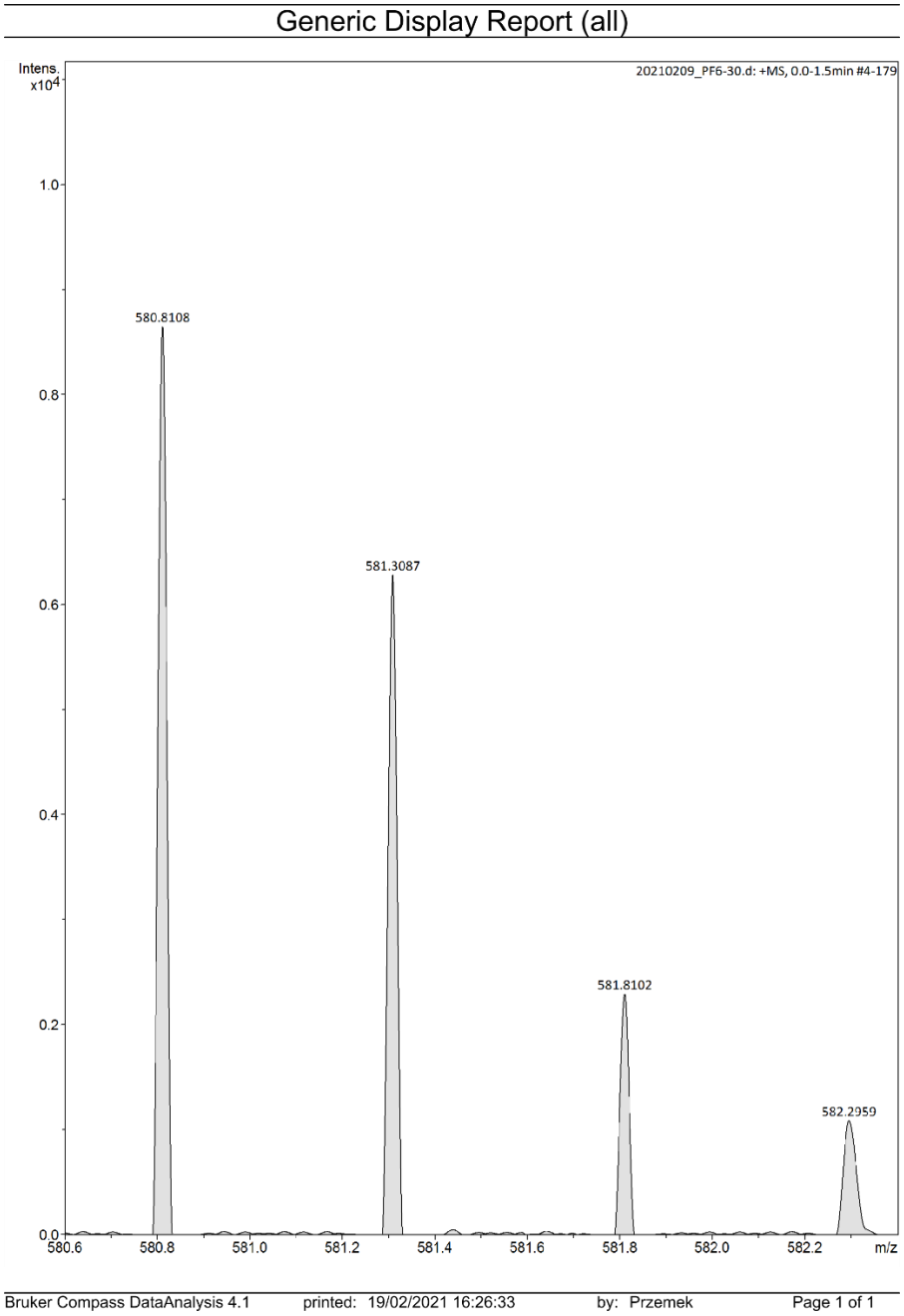


Figure 36: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate synthesized in glassware, and Angiotensin 2

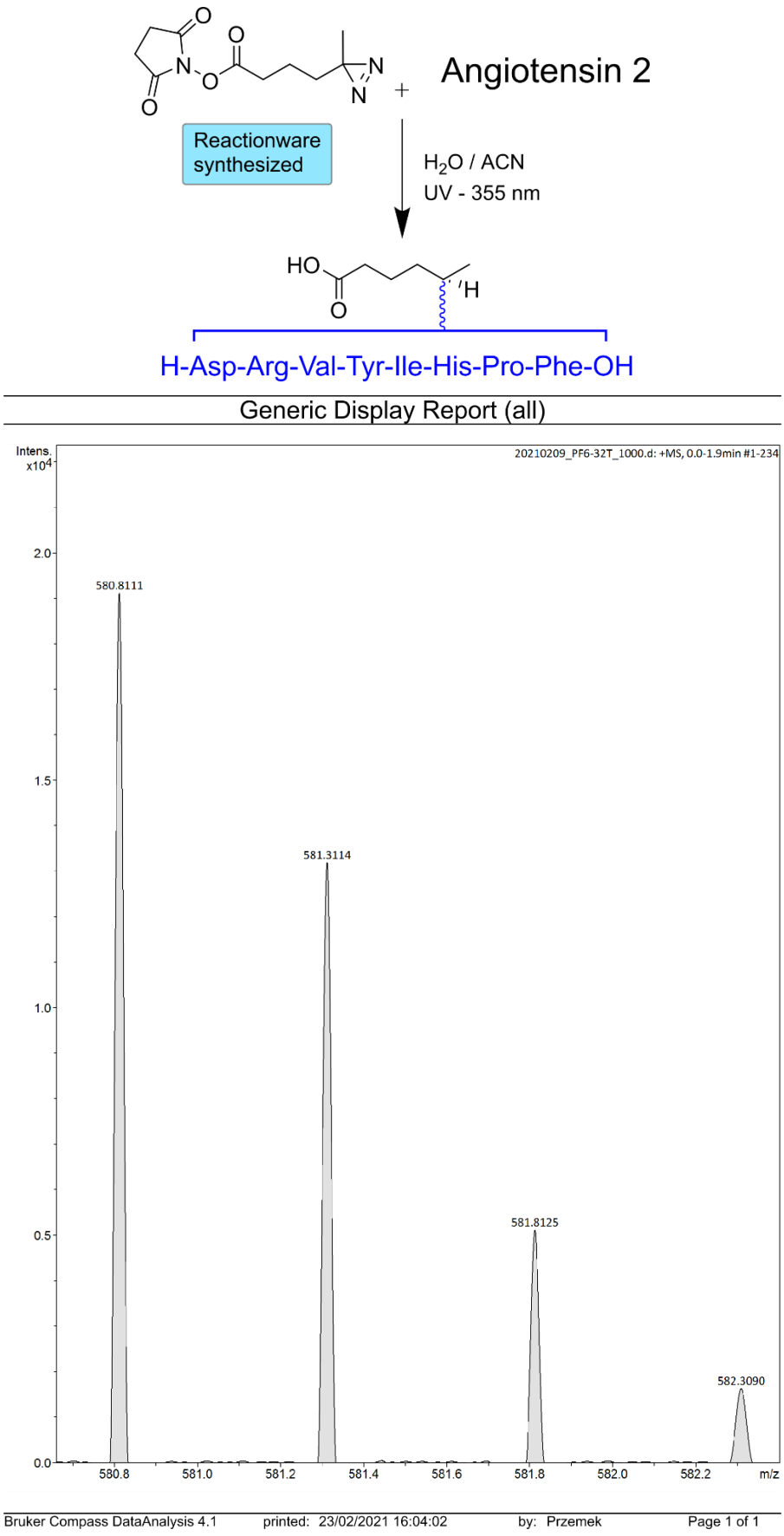
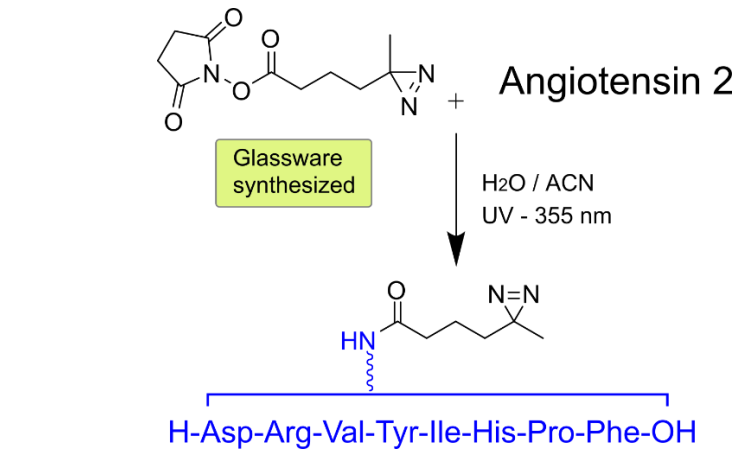


Figure 37: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate synthesized in reactionware, and Angiotensin 2



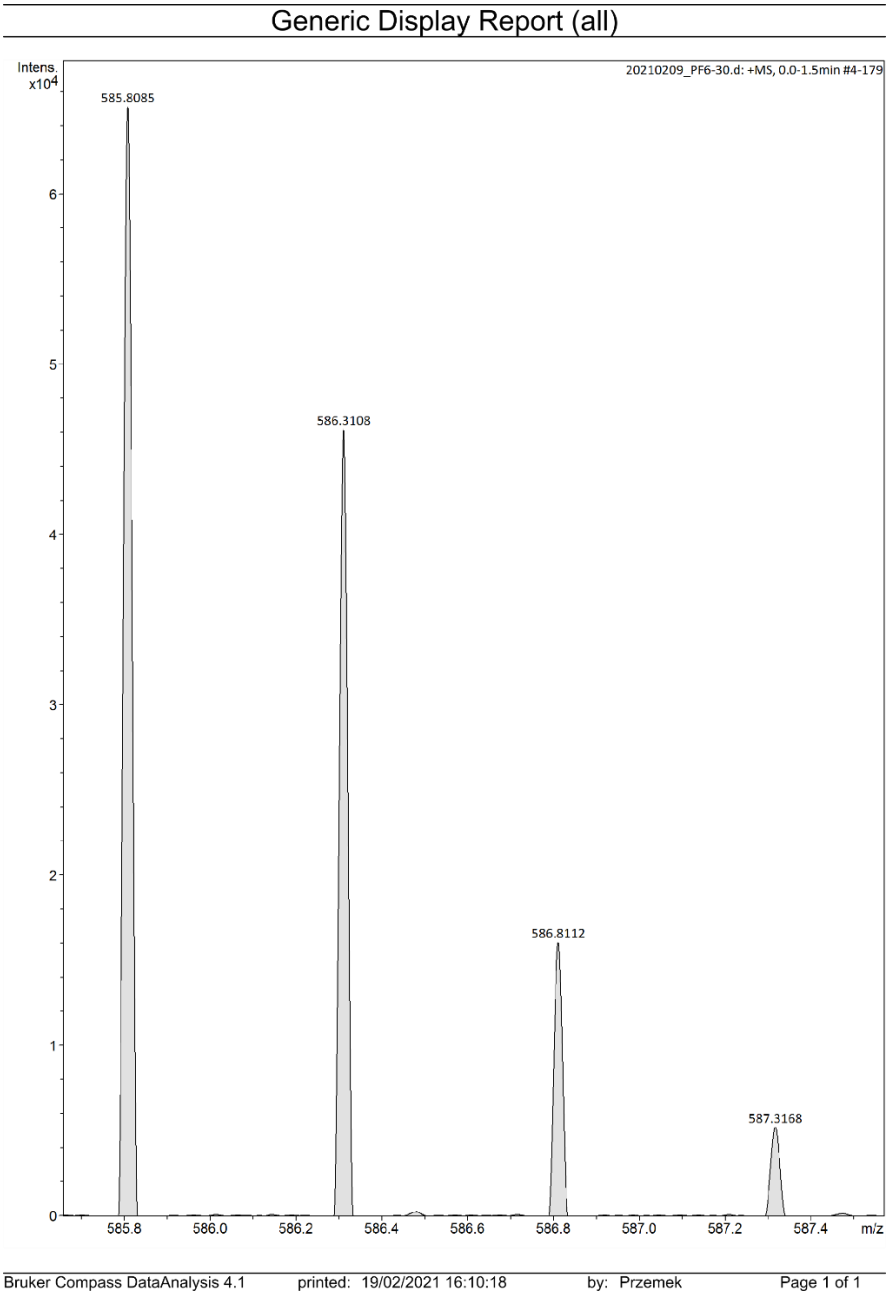


Figure 38: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate synthesized in glassware, and Angiotensin 2

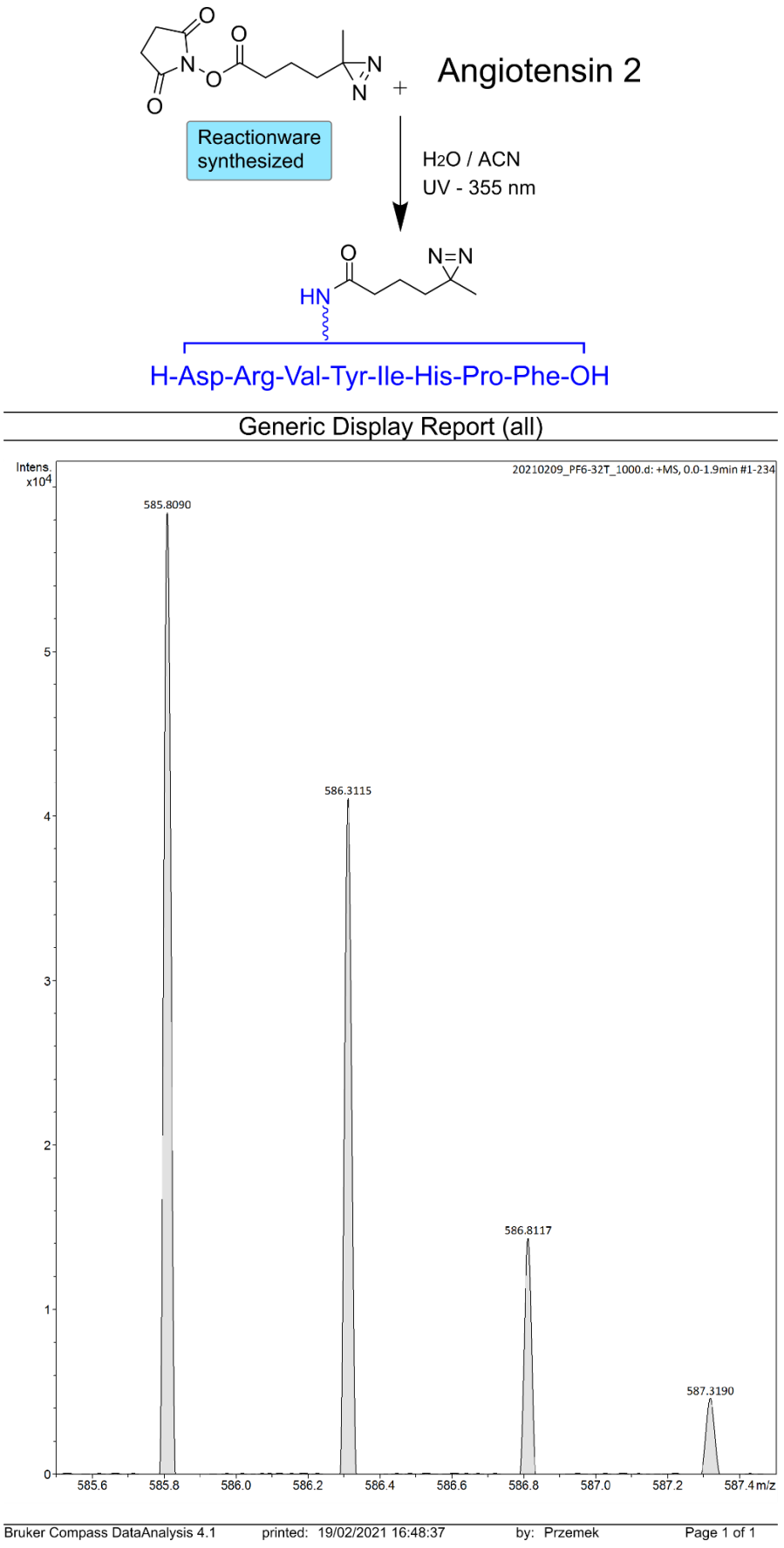


Figure 39: Product formed as a result of reaction between 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate synthesized in reactionware, and Angiotensin 2

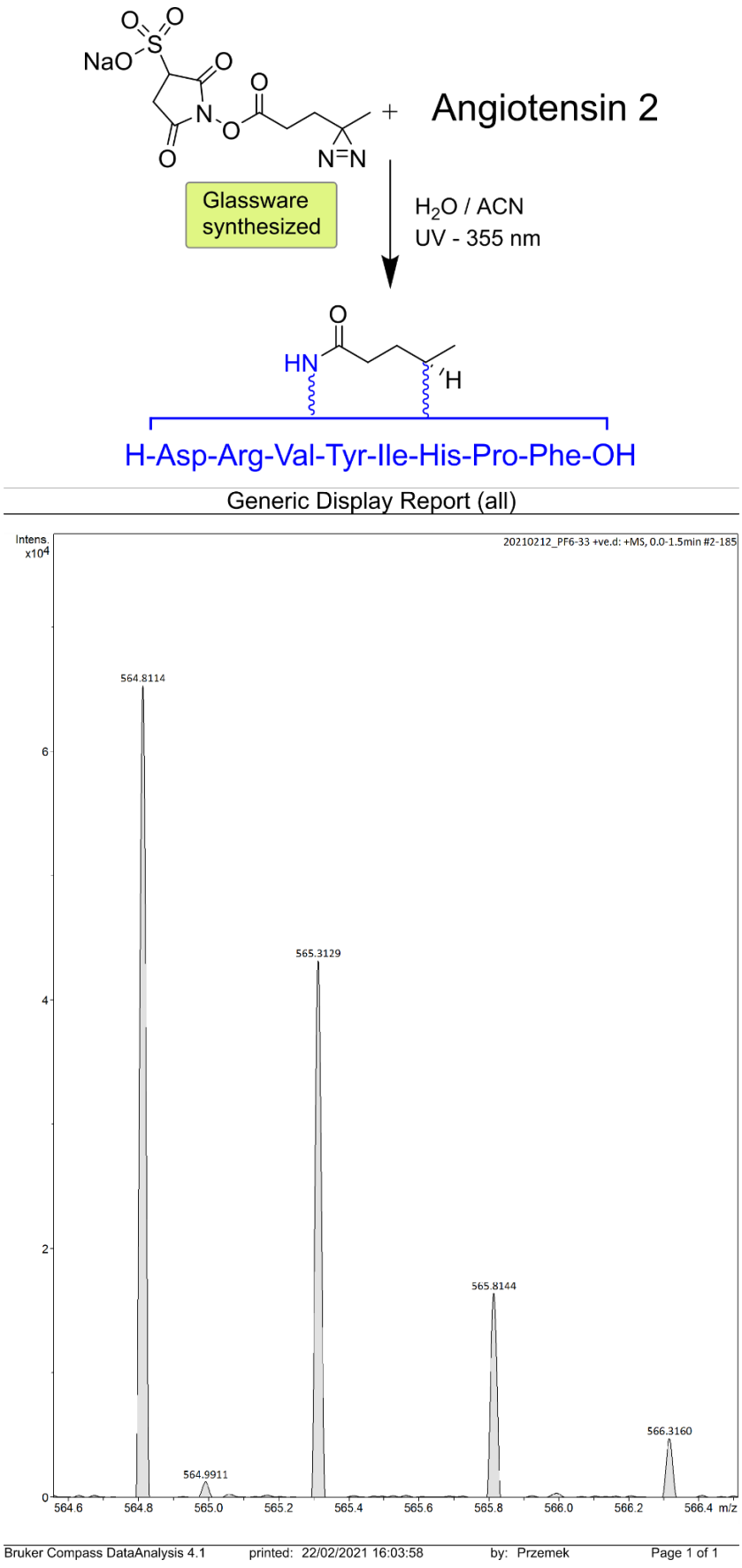


Figure 40: Product formed as a result of reaction between Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate synthesized in glassware, and Angiotensin 2

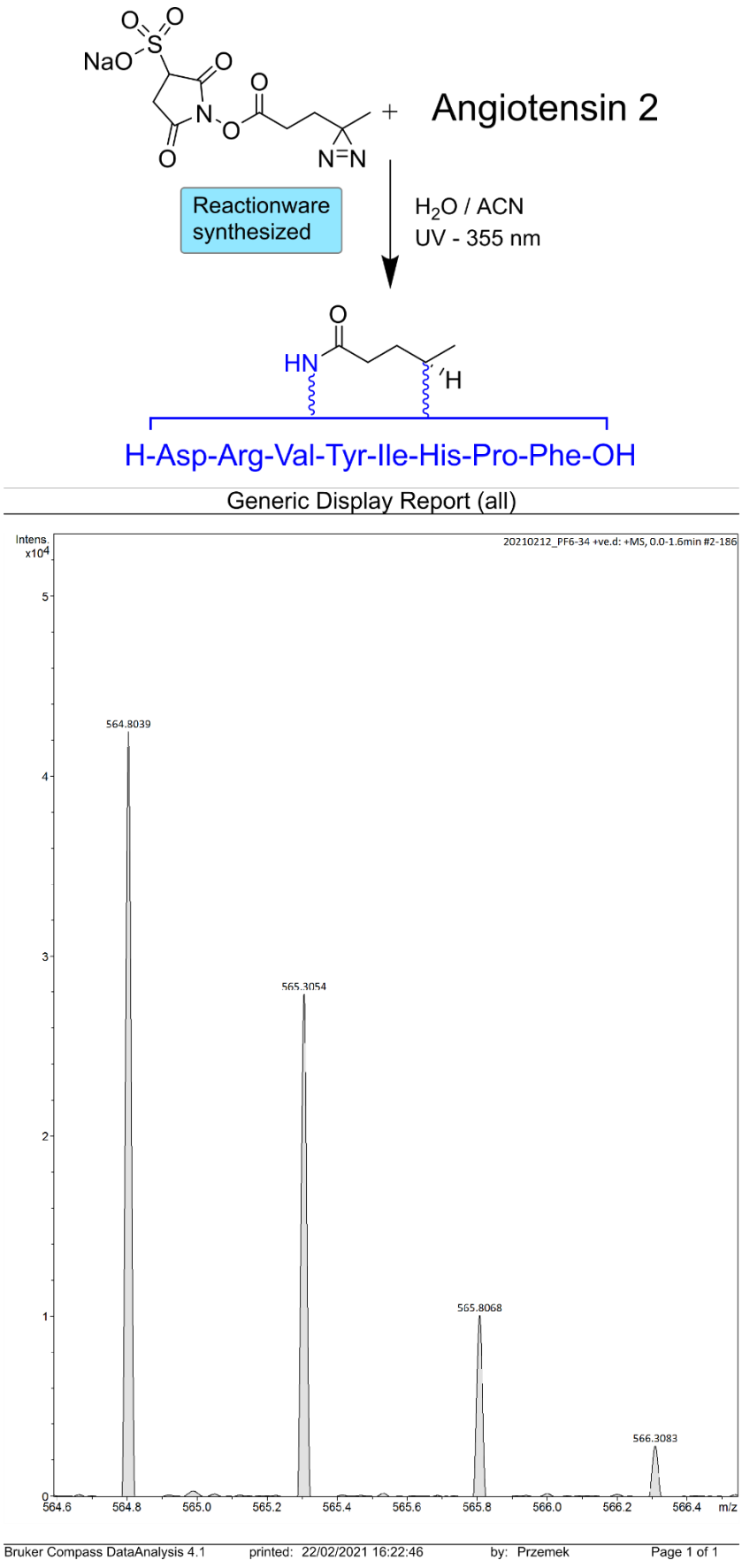


Figure 41: Product formed as a result of reaction between Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate synthesized in reactionware, and Angiotensin 2

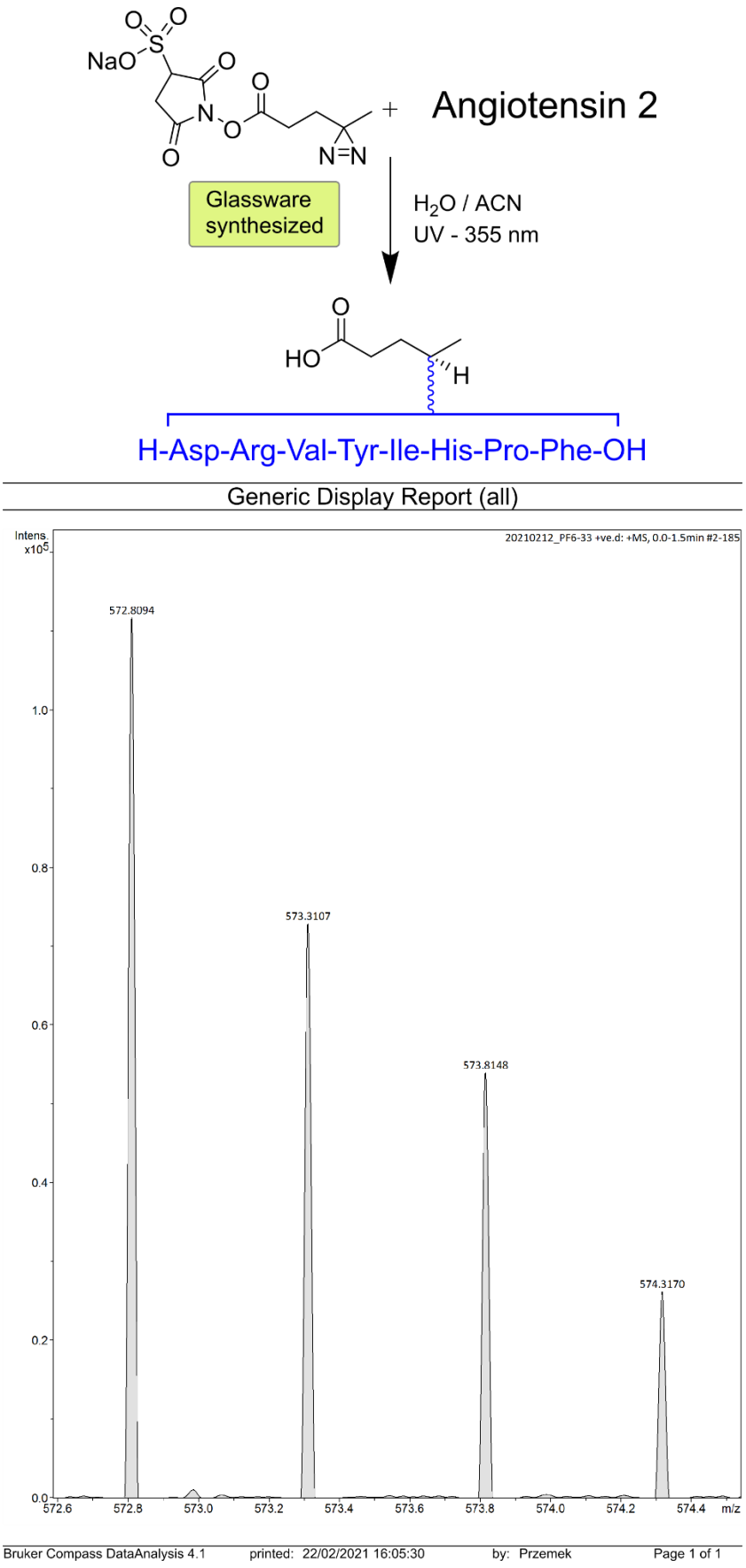


Figure 42: Product formed as a result of reaction between Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate synthesized in glassware, and Angiotensin 2

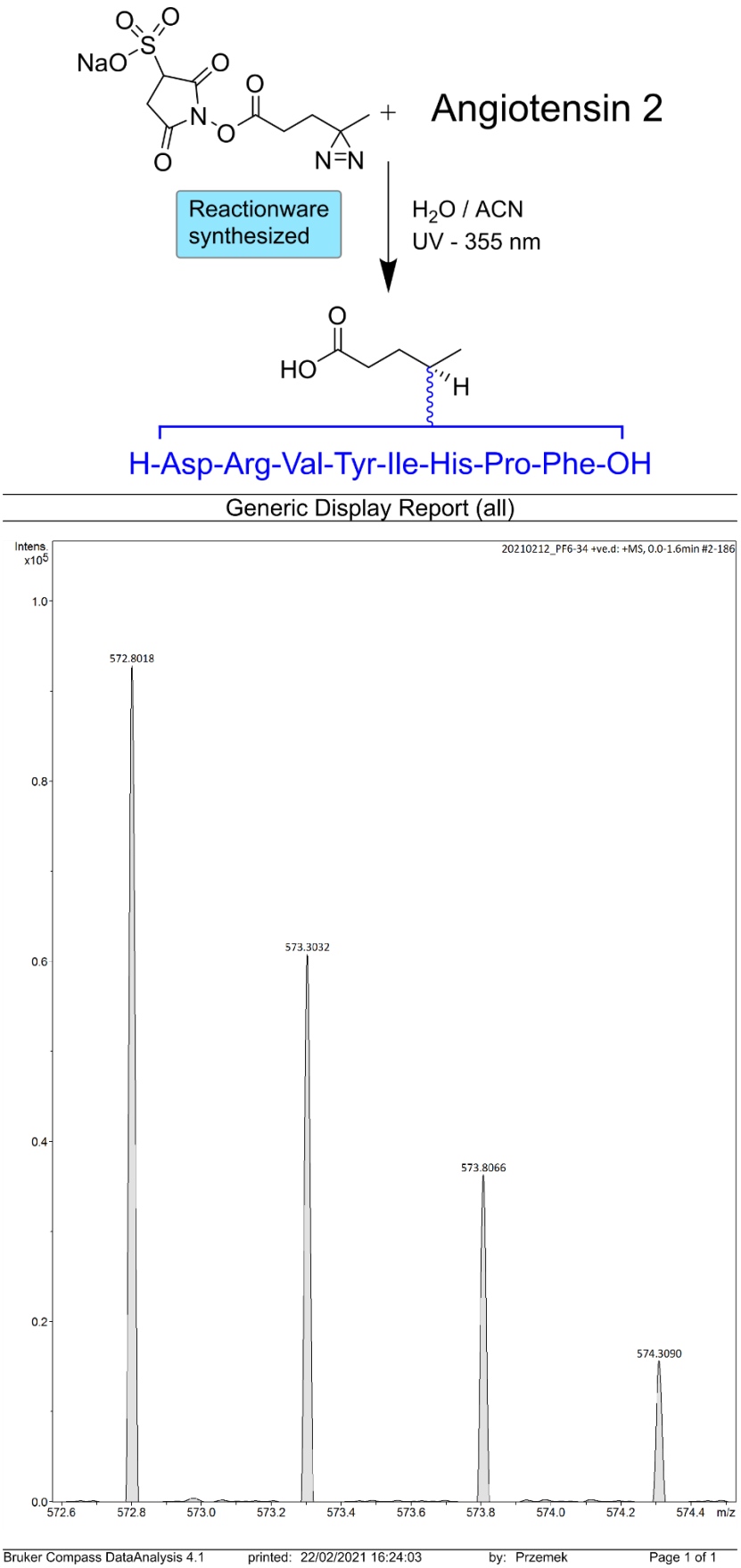


Figure 43: Product formed as a result of reaction between Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate synthesized in reactionware, and Angiotensin 2

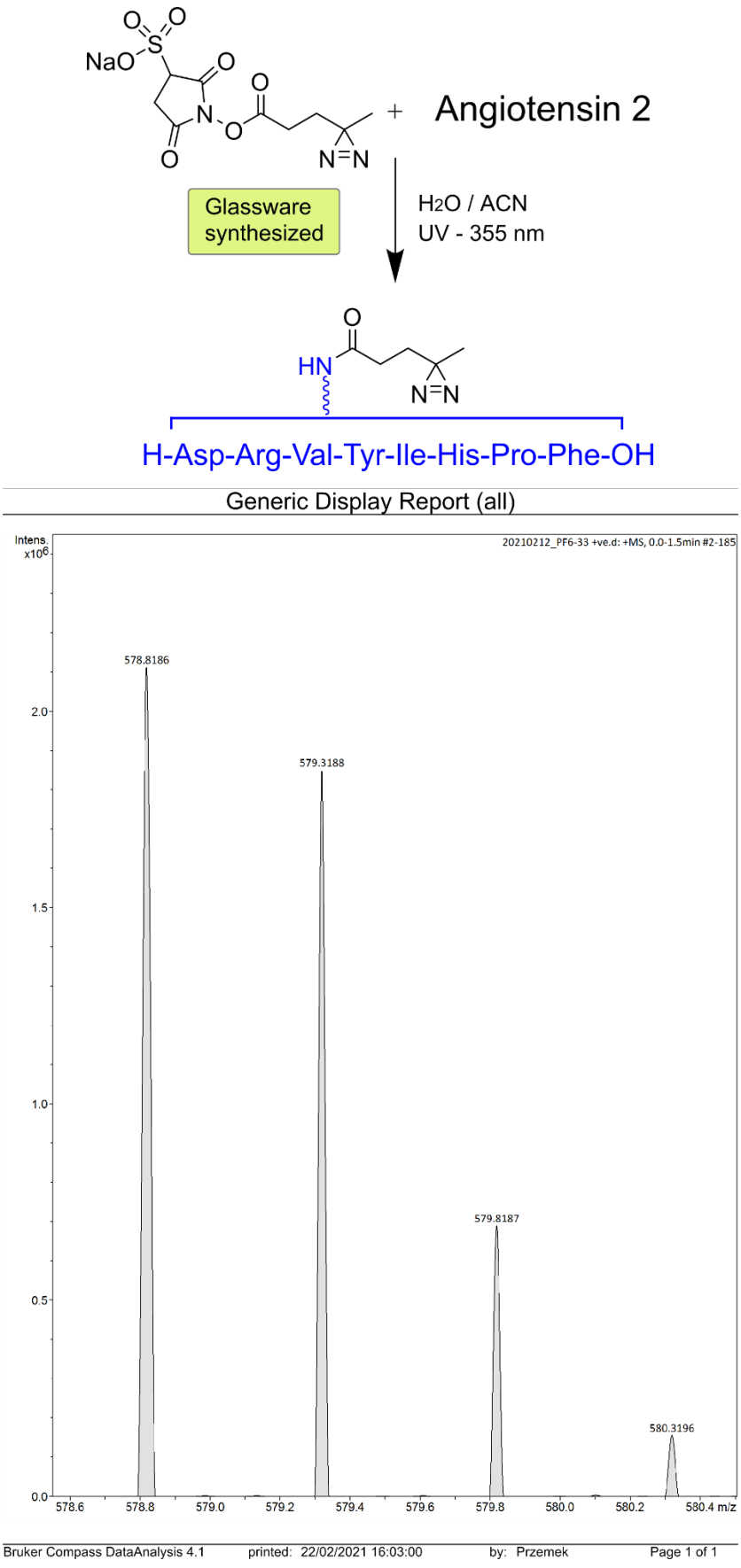


Figure 44: Product formed as a result of reaction between Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate synthesized in glassware, and Angiotensin 2

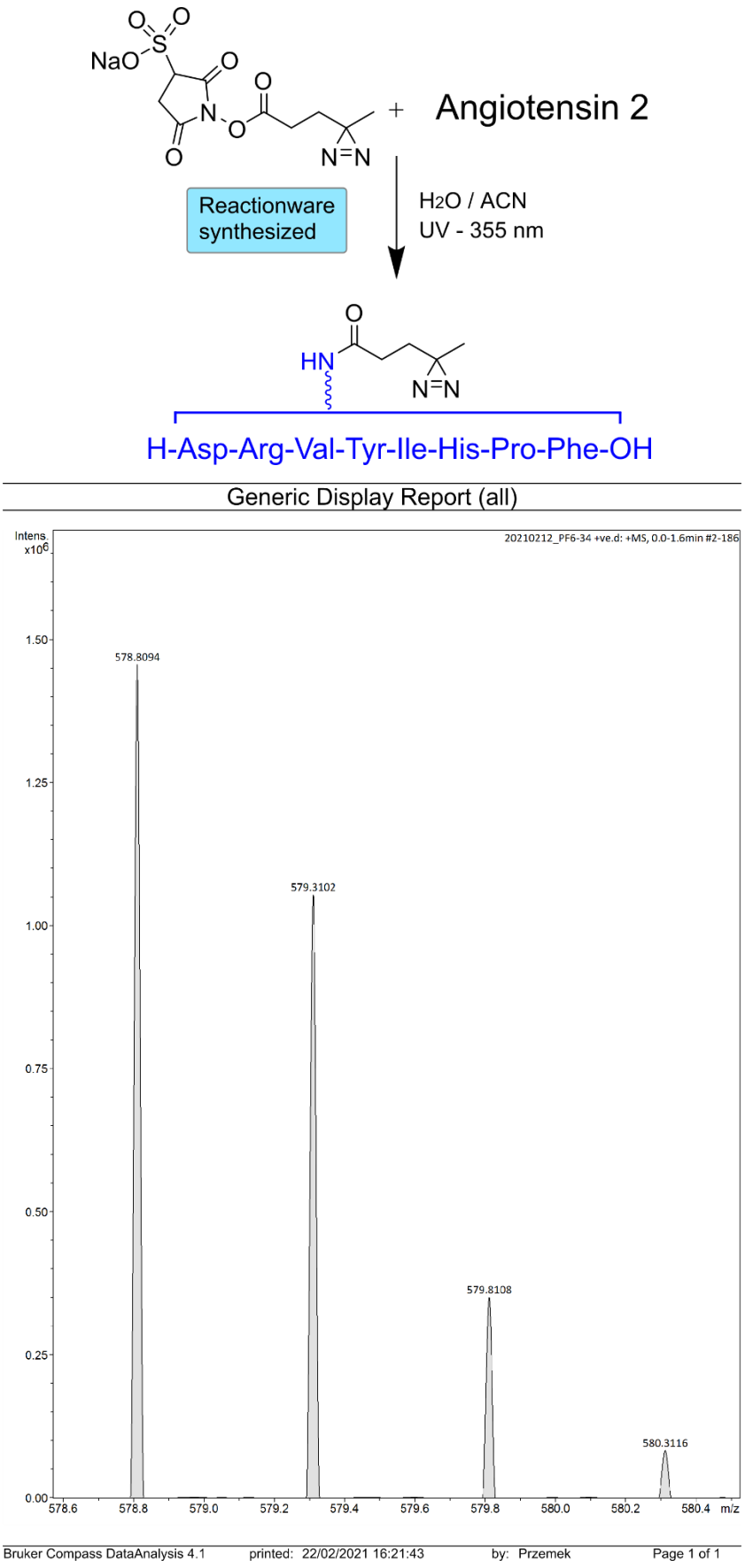


Figure 45: Product formed as a result of reaction between Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate synthesized in reactionware, and Angiotensin 2

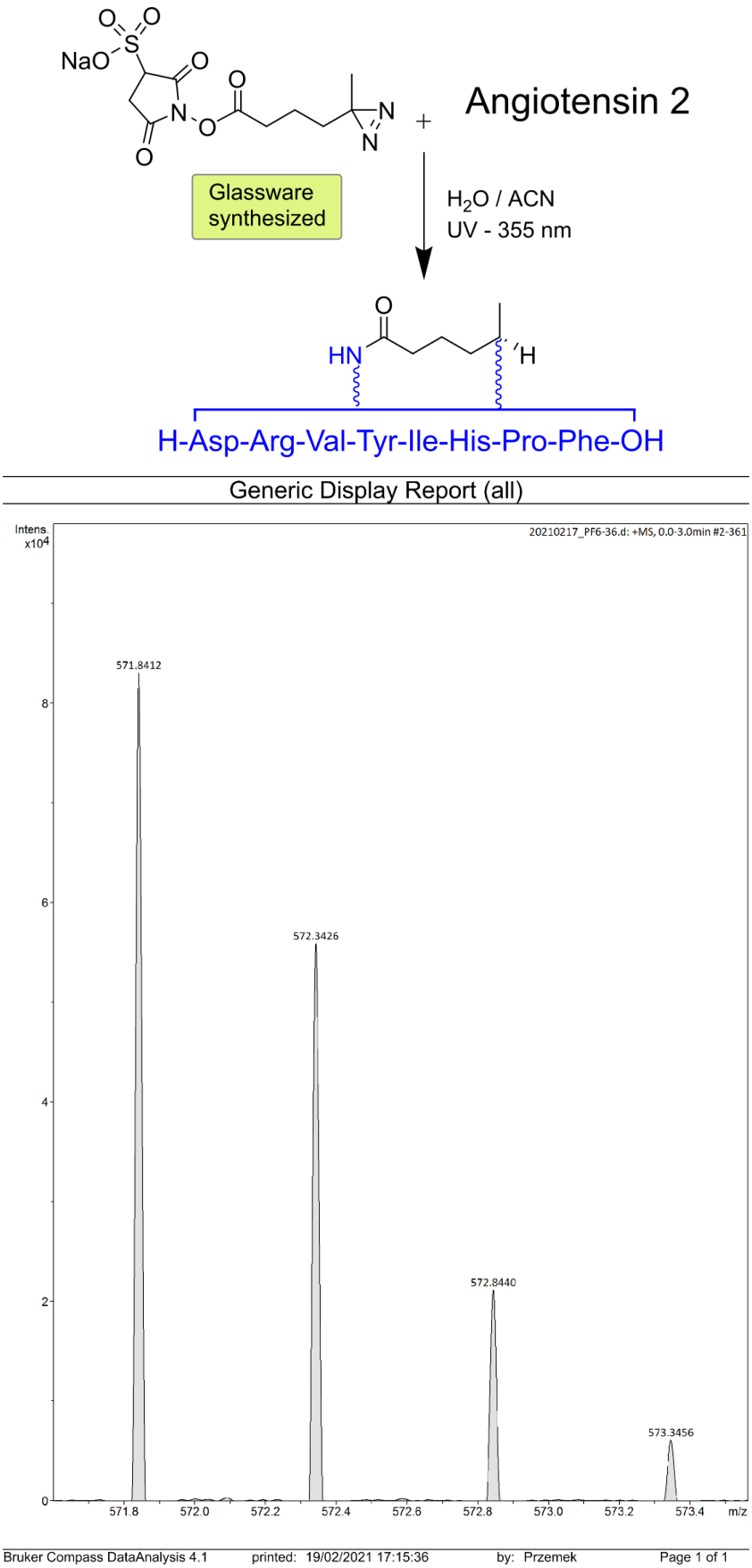


Figure 46: Product formed as a result of reaction between Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate synthesized in glassware, and Angiotensin 2

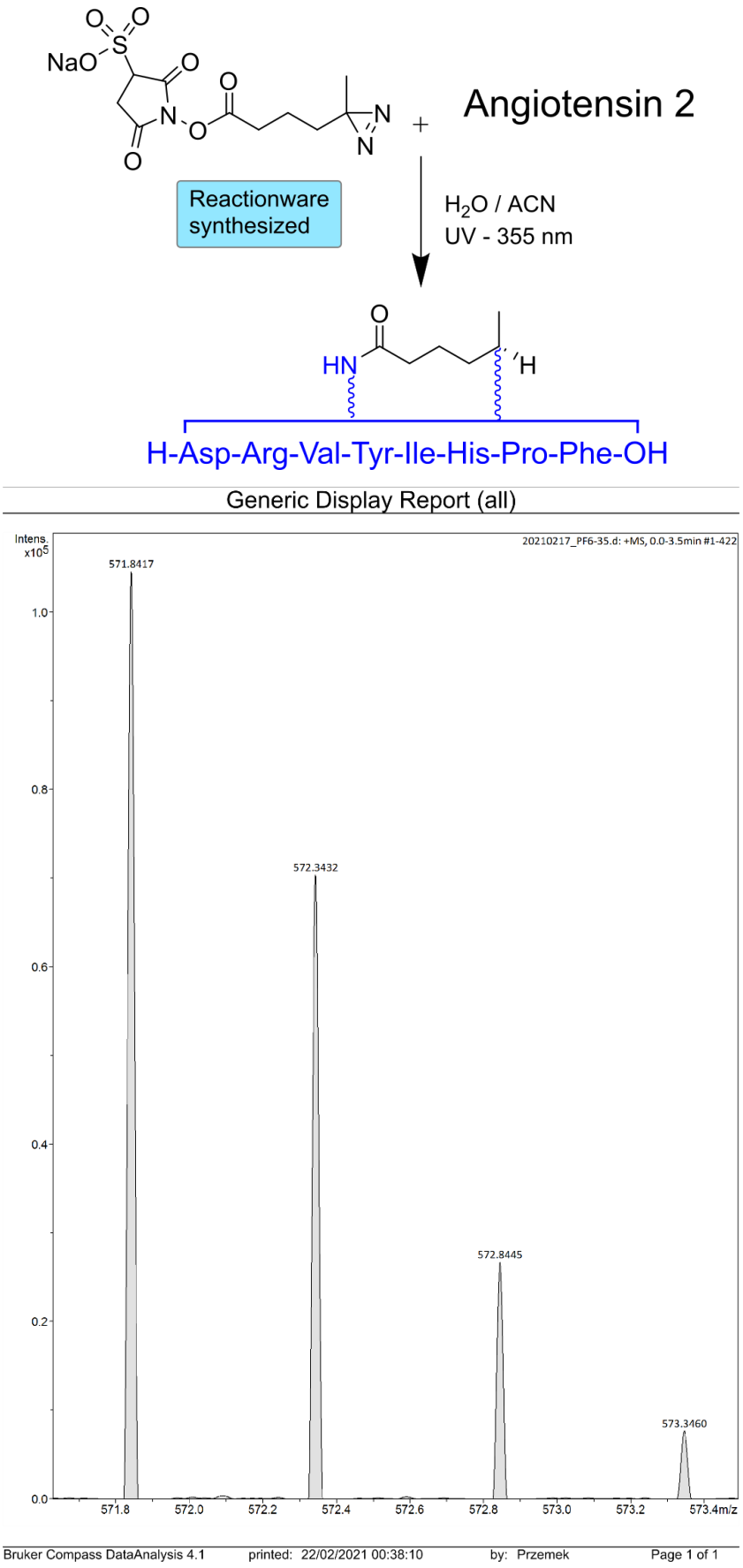


Figure 47: Product formed as a result of reaction between Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate synthesized in reactionware, and Angiotensin 2

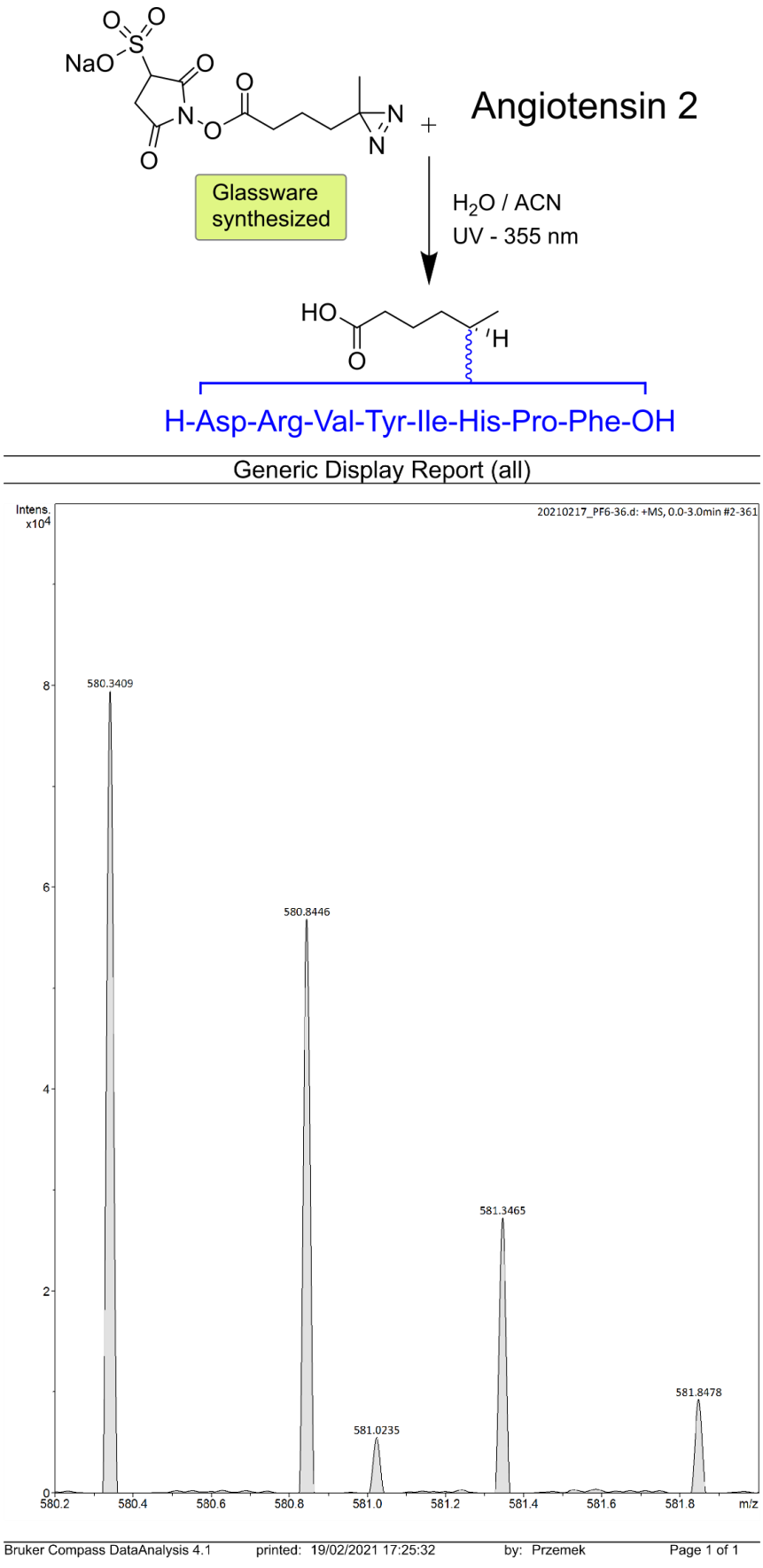


Figure 48: Product formed as a result of reaction between Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate synthesized in glassware, and Angiotensin 2

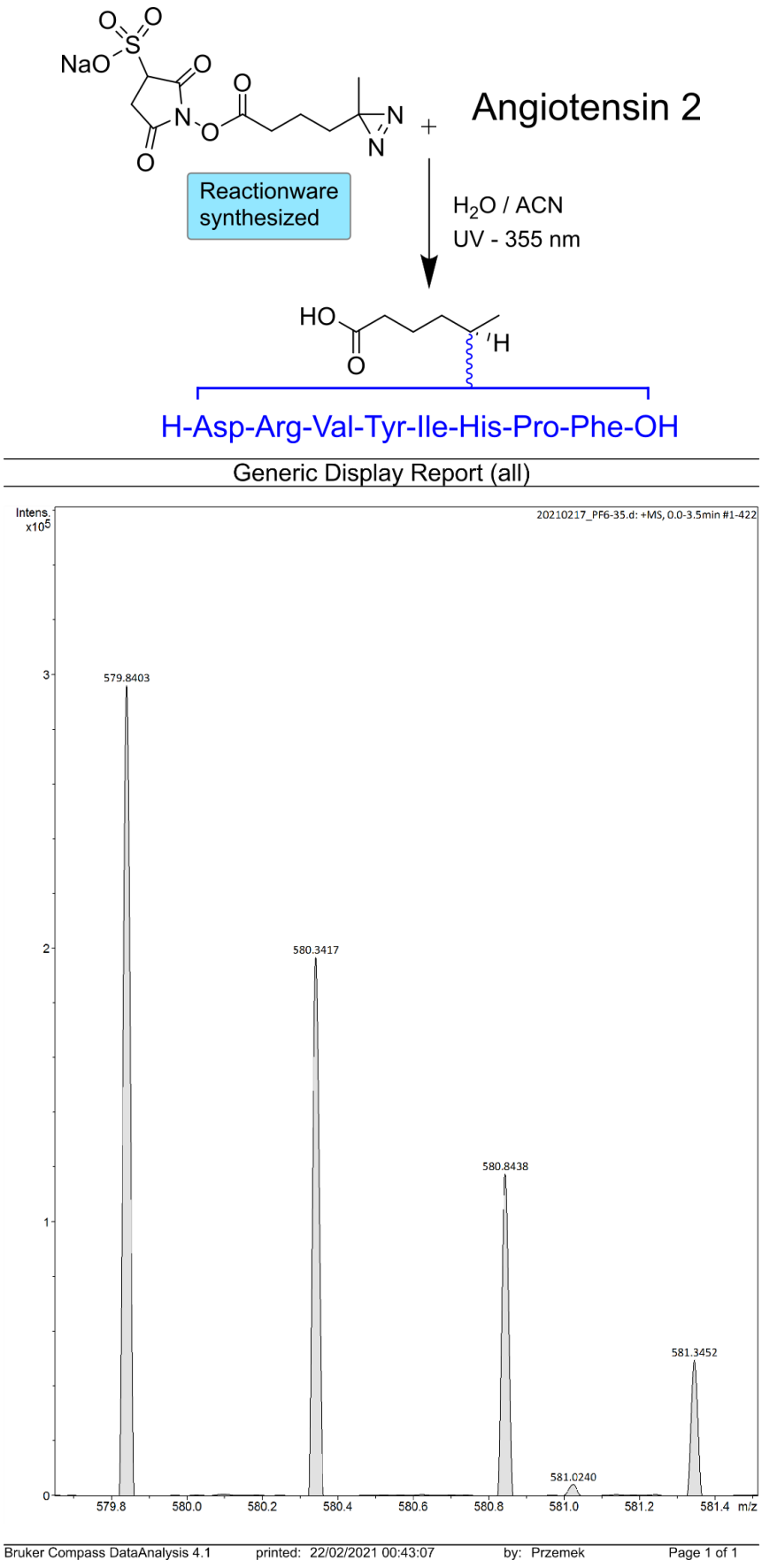


Figure 49: Product formed as a result of reaction between Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate synthesized in reactionware, and Angiotensin 2

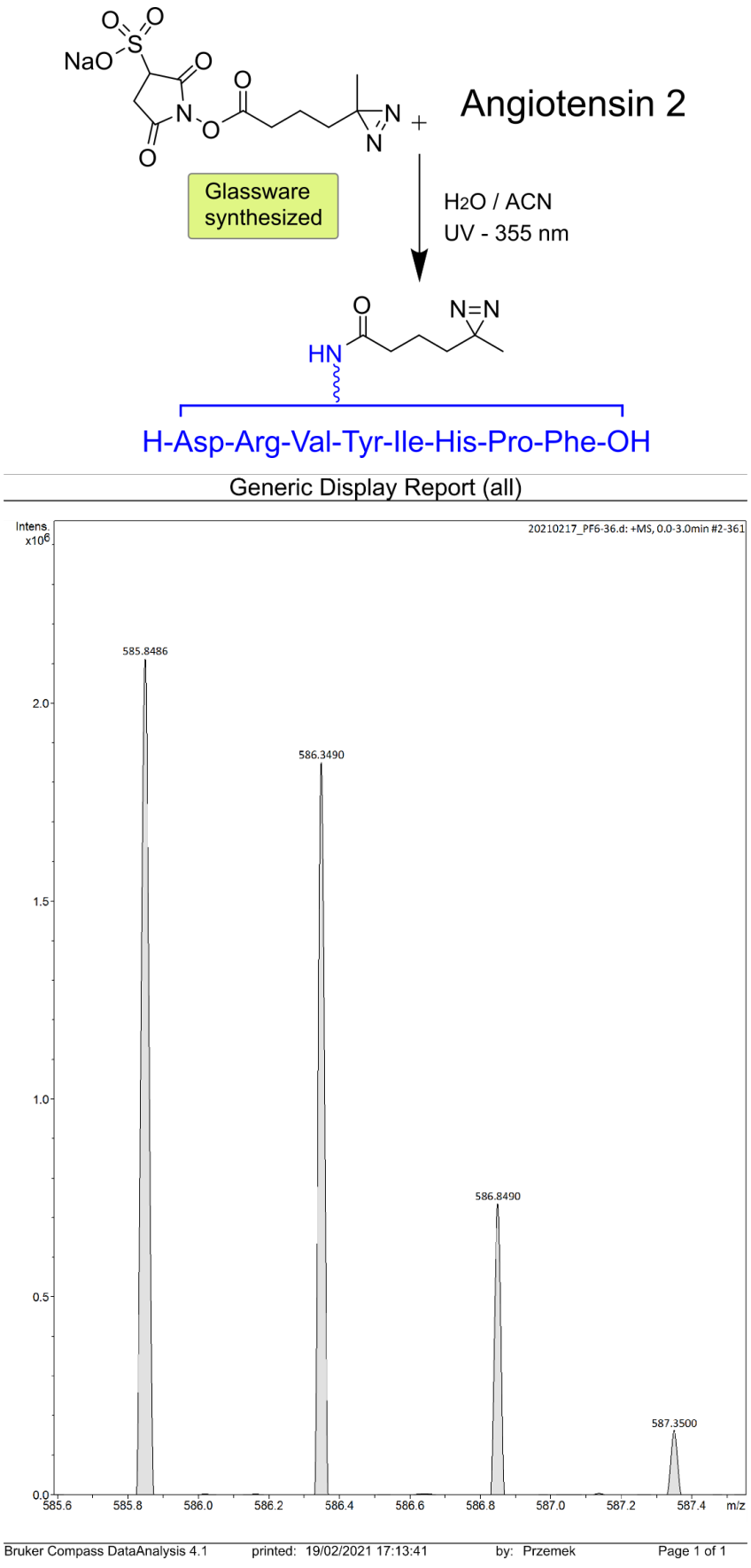


Figure 50: Product formed as a result of reaction between Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate synthesized in glassware, and Angiotensin 2

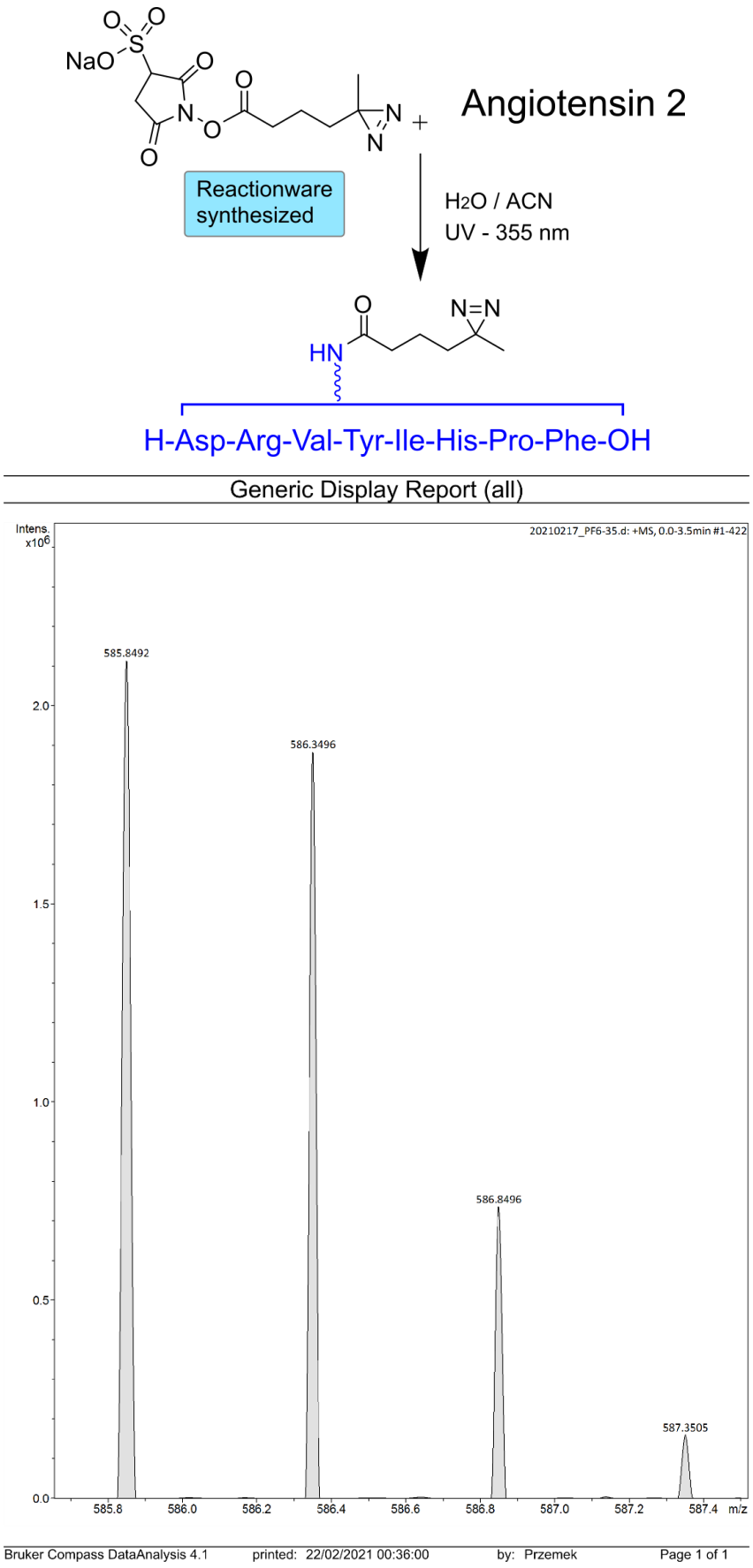


Figure 51: Product formed as a result of reaction between Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate synthesized in reactionware, and Angiotensin 2

4.6.3 Notes on the DEPTQ NMR spectroscopy results

DEPTQ spectra of the reaction mixtures were carried out after reactions between photo-probes and the substrate had taken place. Spectra obtained from experiments that utilized photo-probes synthesized in glassware were compared against those utilizing photo-probes synthesized in reactionware. Crucially those were also compared to the spectra obtained from the control experiments carried out either without a photo-probe or without the substrate. We anticipated that if no new species were formed, the spectral patterns obtained from each experimental run would remain largely similar to those of the control spectra added together. Instead we observed areas where peak patterns were significantly altered (parts of spectrums highlighted in red), suggesting that at least portion the substrate changed its electronic arrangement as a result of a covalent bonding with the photo-probes.

4.6.4 DEPTQ NMR results from validation experiments.

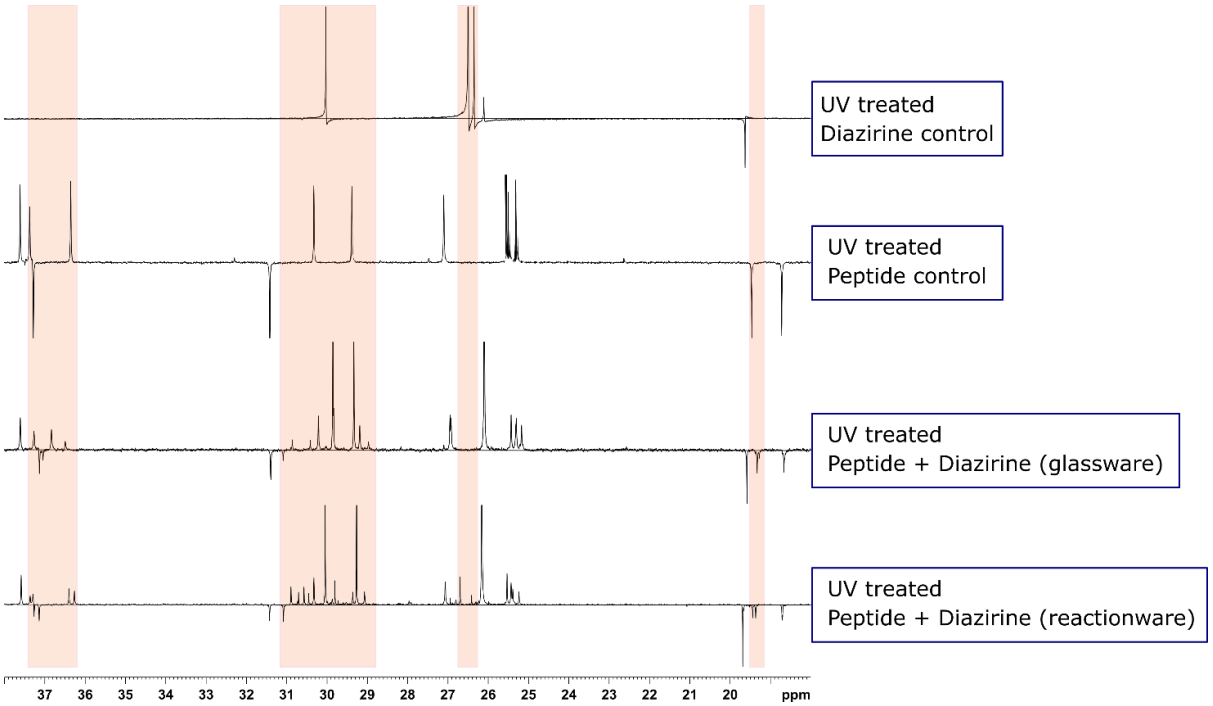


Figure 52: Comparison of DEPTQ NMR spectra from experiments carried out with 2,5-dioxopyrrolidin-1-yl 3-(3-methyl-3H-diazirin-3-yl)propanoate.

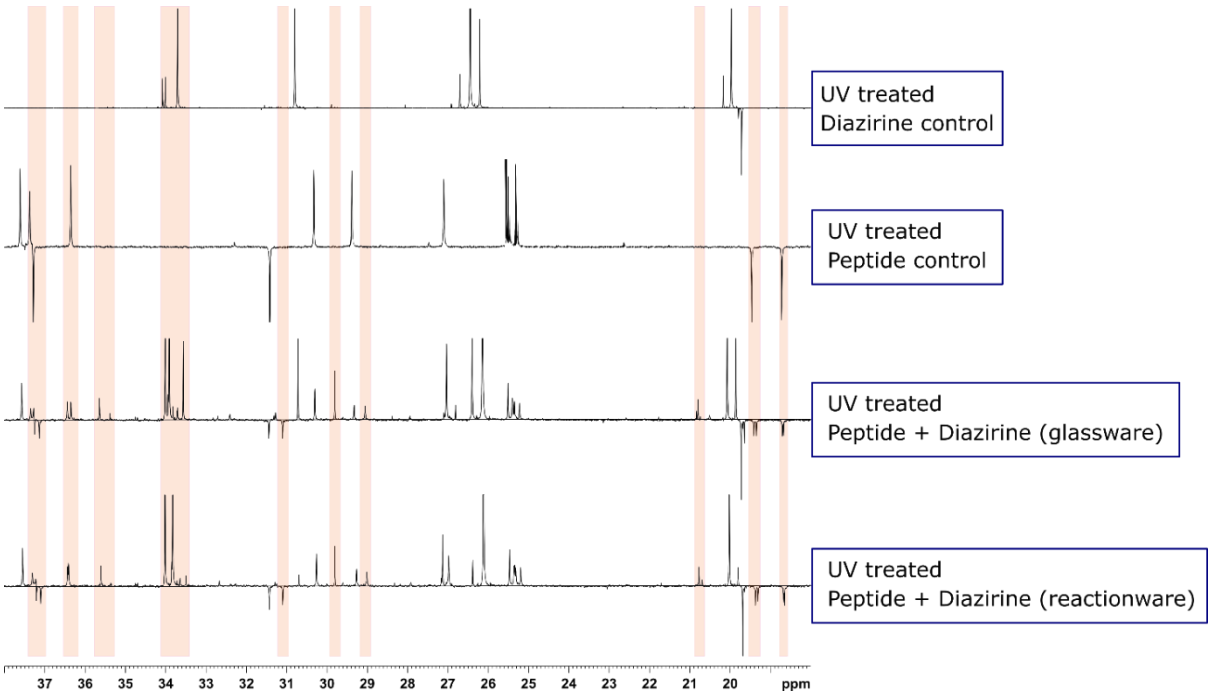


Figure 53: Comparison of DEPTQ NMR spectra from experiments carried out with 2,5-dioxopyrrolidin-1-yl 4-(3-methyl-3H-diazirin-3-yl)butanoate

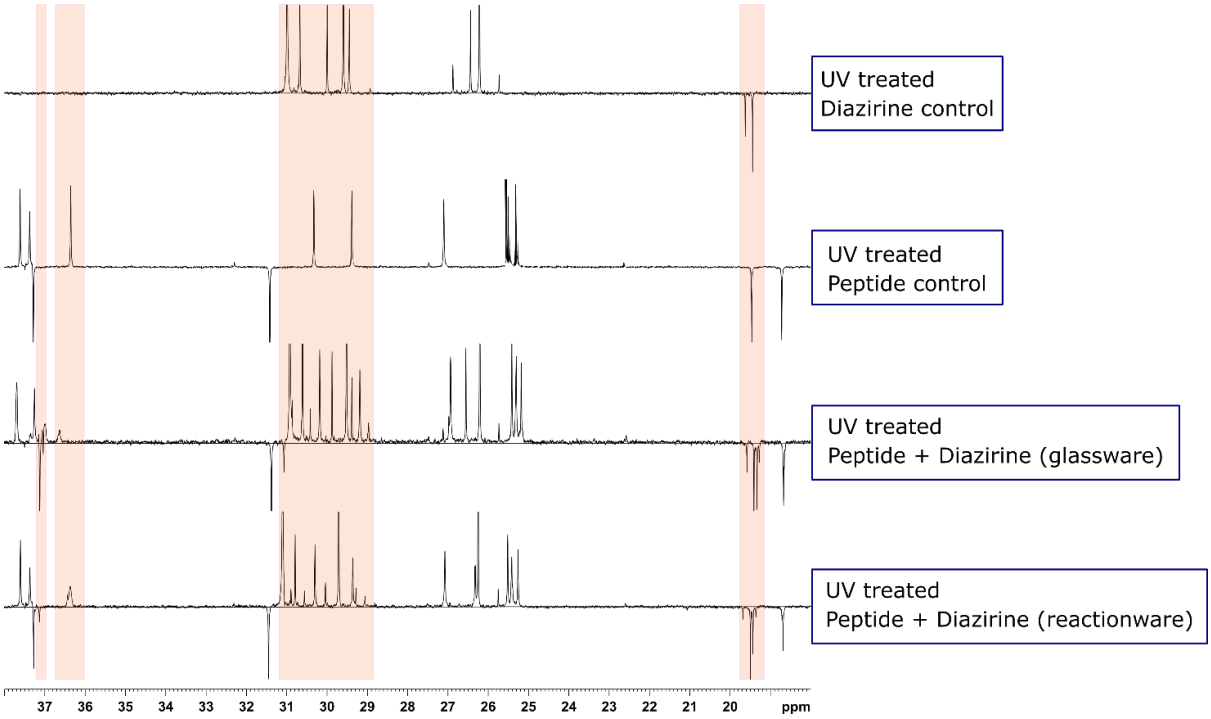


Figure 54: Comparison of DEPTQ NMR spectra from experiments carried out with Sodium 1-{[3-(3-methyl-3H-diazirin-3-yl)propanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate

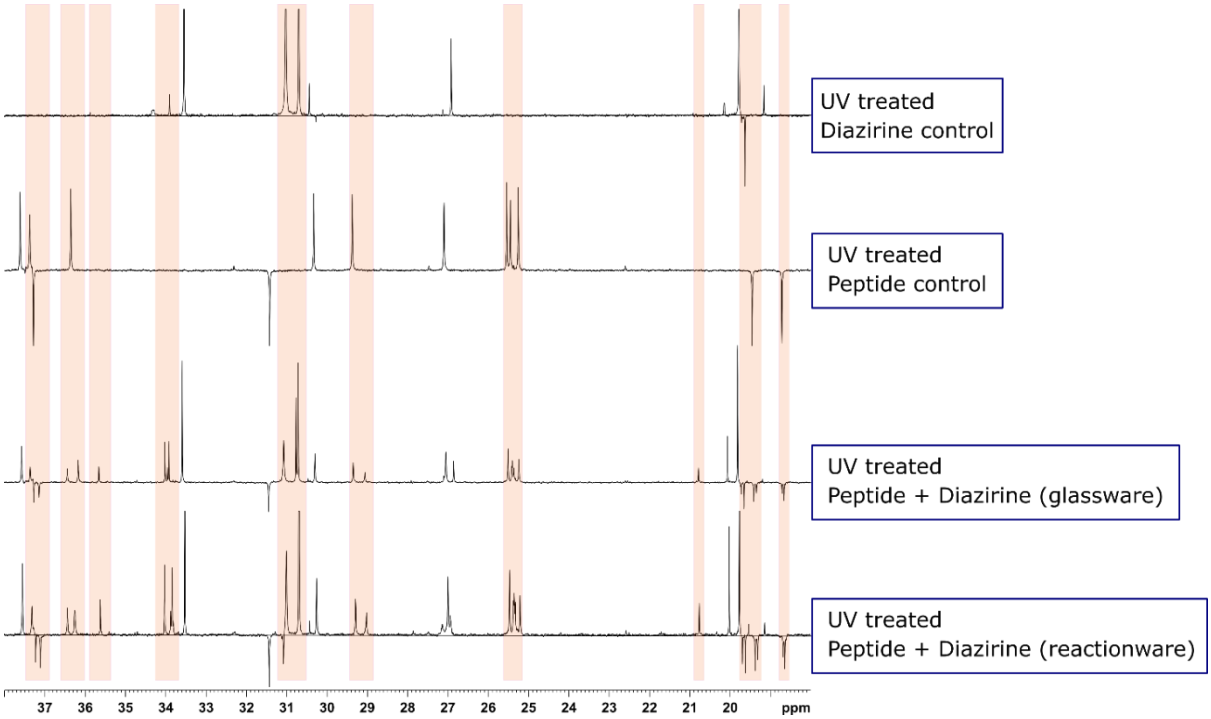


Figure 55 : Comparison of DEPTQ NMR spectra from experiments carried out with Sodium 1-{[4-(3-methyl-3H-diazirin-3-yl)butanoyl]oxy}-2,5-dioxopyrrolidine-3 sulfonate

5 References

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