

Supporting Information

Rapid discovery of potent α -fucosidase inhibitors by *in situ* screening of a library of (pyrrolidin-2-yl)triazoles

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and Inmaculada Robina

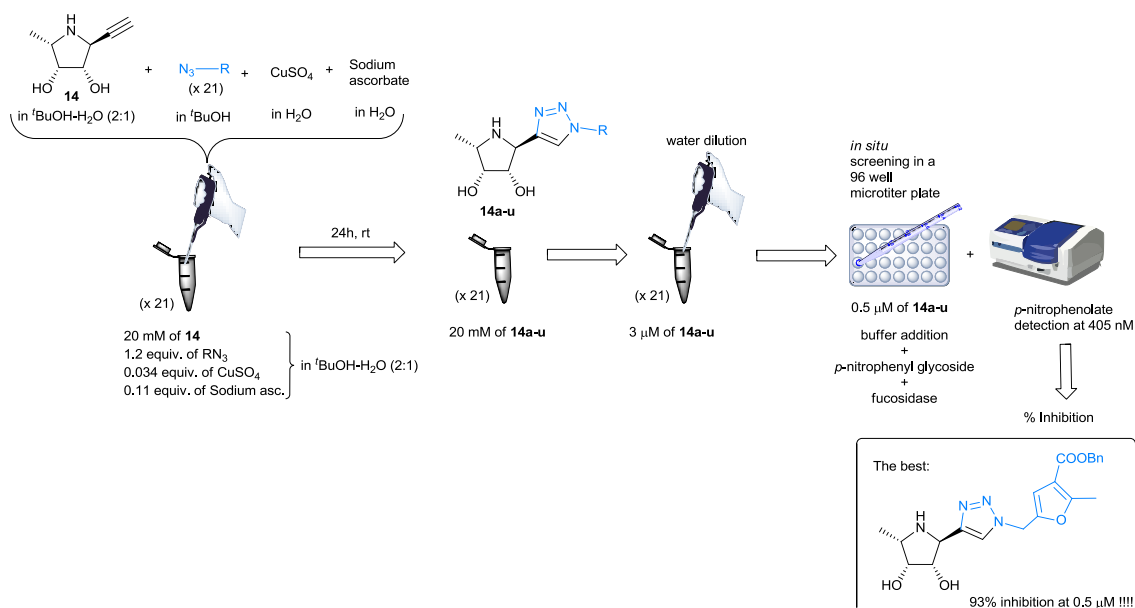
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1. Detailed scheme for *in situ* screening towards α -fucosidases from bovine kidney



2. IC₅₀ and Lineweaver-Burk plots

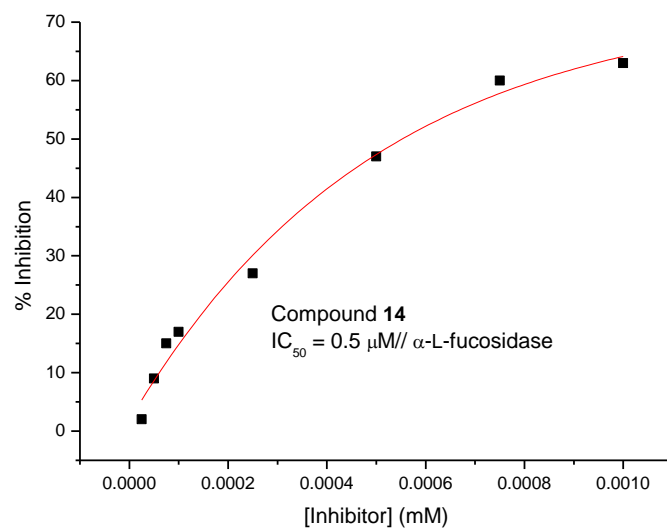


Figure 1. IC₅₀ of compound **14** towards α-L-fucosidase (bovine kidney)

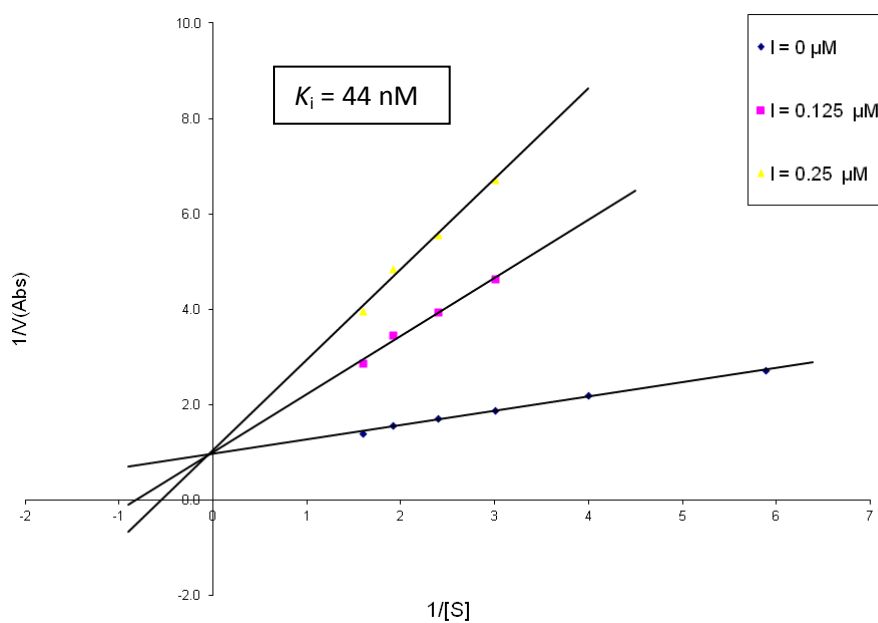


Figure 2. K_i of compound **14** towards α-L-fucosidase (bovine kidney)

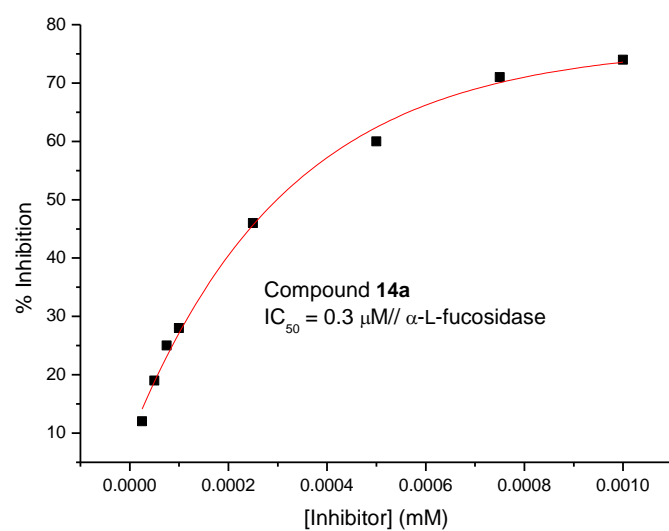


Figure 3. IC_{50} of compound **14a** towards α -L-fucosidase (bovine kidney)

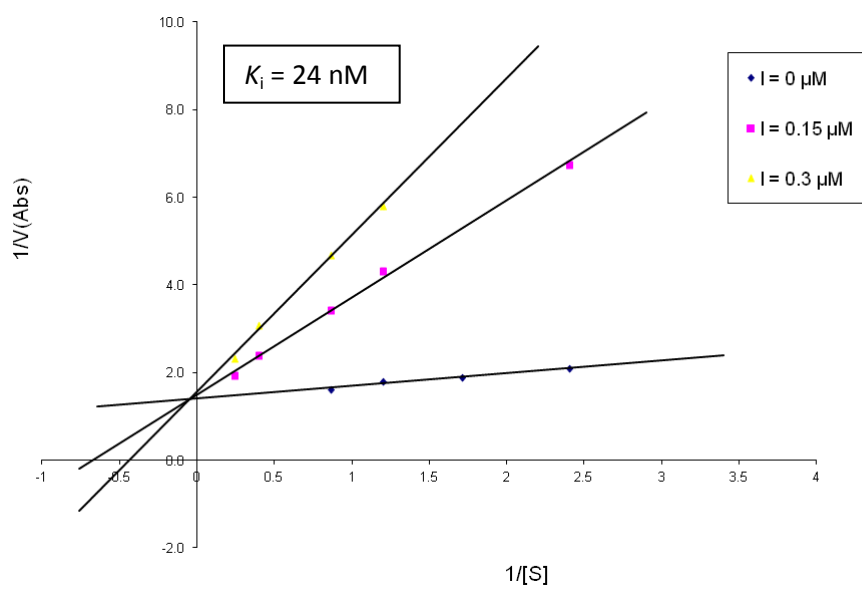


Figure 4. K_i of compound **14a** towards α -L-fucosidase (bovine kidney)

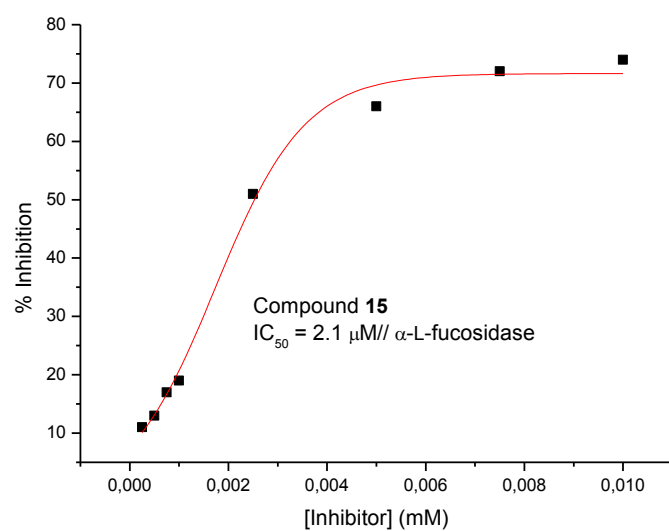


Figure 5. IC_{50} of compound **15** towards α -L-fucosidase (bovine kidney)

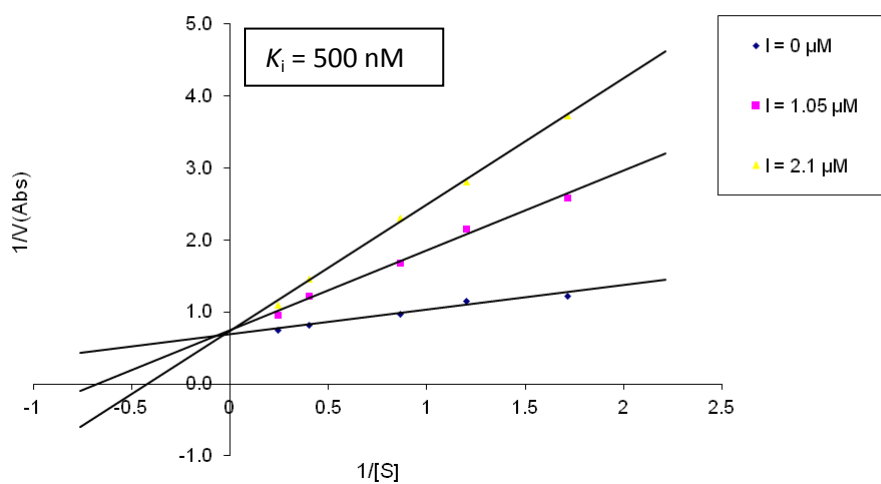


Figure 6. K_i of compound **15** towards α -L-fucosidase (bovine kidney)

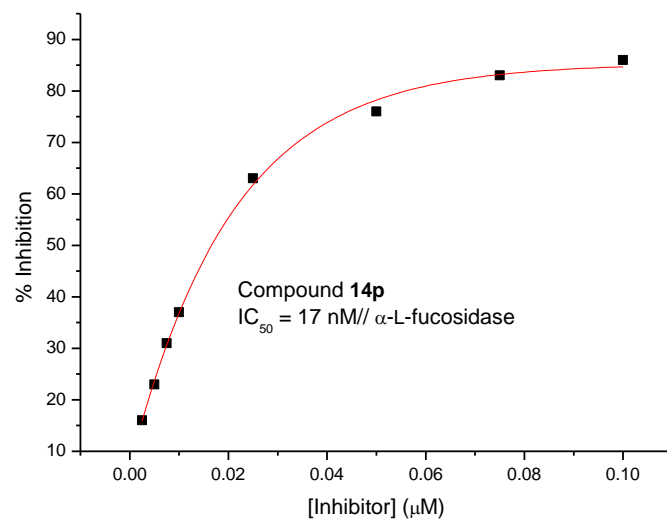


Figure 7. IC_{50} of compound **14p** towards α -L-fucosidase (bovine kidney)

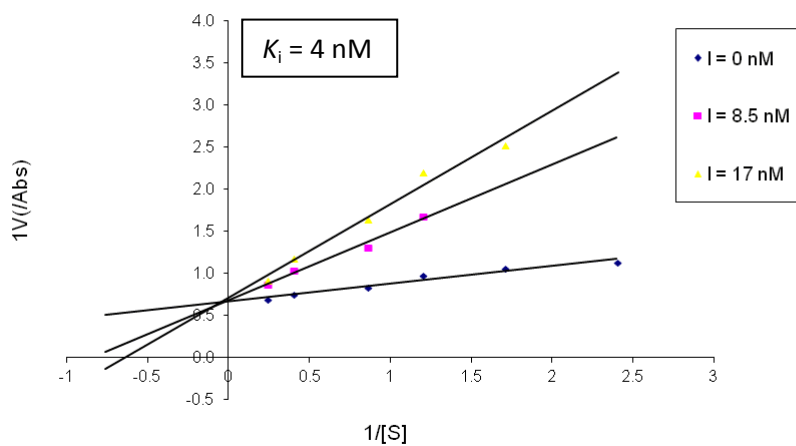
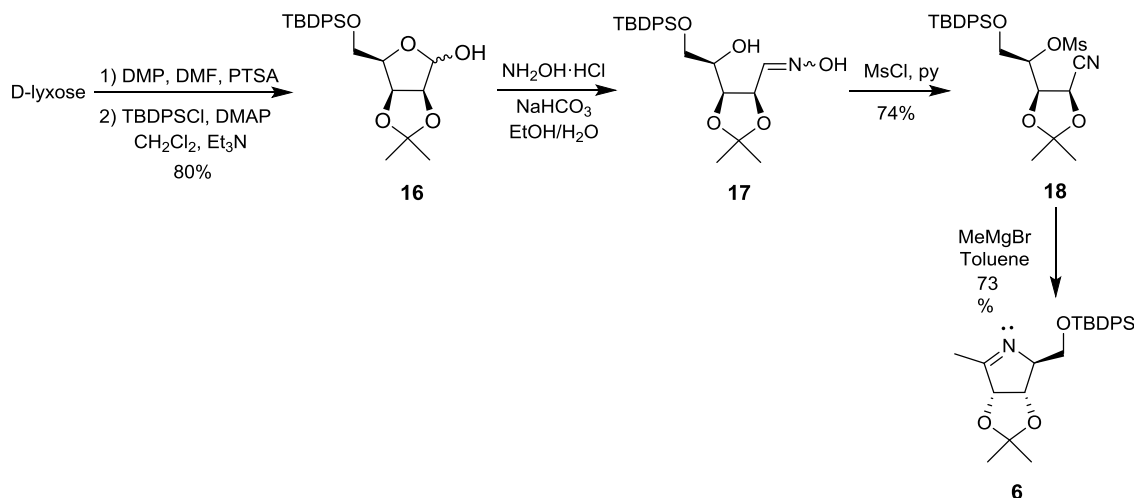


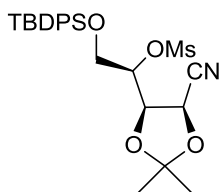
Figure 8. K_i of compound **14p** towards α -L-fucosidase (bovine kidney)

3. Experimental details for the preparation of compound 6

Synthesis of pyrroline 6^{1,2}



5-*O*-*tert*-Butyldiphenylsilyl-2,3-*O*-isopropylidene-4-*O*-methanesulfonyl-D-lyxonitrile (18)



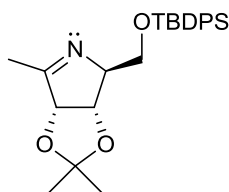
To a solution of hydroxylamine hydrochloride (7.5 g, 104.7 mmol) in EtOH:H₂O (1:1, 160 mL), NaHCO₃ (8.8 g, 104.7 mmol) was slowly added. The solution was stirred at r.t. for 15 min. Then, a solution of compound **16**² (9.96 g, 23.2 mmol) in ethanol (80 mL) was added and the mixture stirred for 72 h at r.t. After concentration under reduced pressure, the residue was diluted with EtOAc and washed with H₂O. The aqueous phase was extracted twice with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give the corresponding oximes **17** which were used in the next step without purification. Methanesulfonylchloride (15 mL, 193.8 mmol) was slowly added to a solution of both oximes in anhydrous pyridine (60 mL) at 0 °C. After 6 h at r.t., the mixture was cooled to 0 °C, H₂O (20 mL) was added and the reaction stirred for 15 min at r.t. Then, the solvent was evaporated, the crude was diluted with EtOAc and washed twice with H₂O. The organic phase was dried over Na₂SO₄, filtered and concentrated. The resulting residue was purified by chromatography column on silica gel (EtOAc/cyclohexane, 1:6) to give **18** (8.6 g, 17.1 mmol, 74%, two steps) as a yellow oil. [α]_D²⁷ + 1.8 (*c* 1.03, CH₂Cl₂). IR (ν cm⁻¹) 2933,

¹ The synthesis of pyrroline **6** was carried out from D-lyxose following the procedure reported by Behr but using the *tert*-butyldiphenylsilyl ether as protecting group: J.-B. Behr, A. Kalla, C. Harakat, R. Plantier-Royon, *J. Org. Chem.*, 2008, **73**, 3612.

² X. Cheng, N. Khan, D. R. Mootoo, *J. Org. Chem.*, 2000, **65**, 2544.

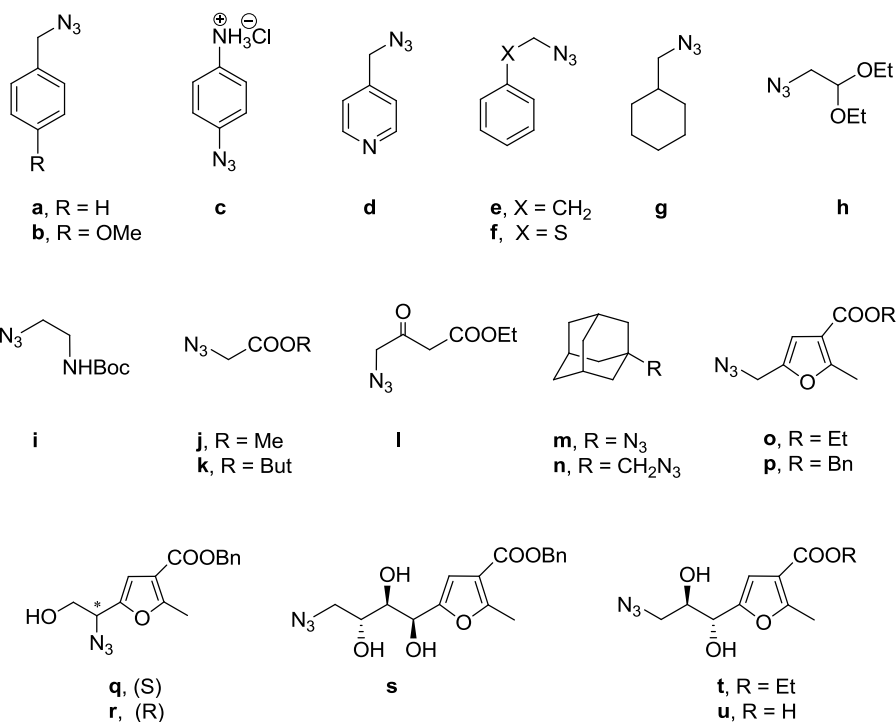
2855, 2387 (CN), 1362, 1177, 702. ^1H NMR (300 MHz, CDCl_3 , δ ppm, J Hz) δ 7.68-7.63 (m, 4H, H-aromat.), 7.48-7.39 (m, 6H, H-aromat.), 4.94 (ddd, 1H, $J_{4,3} = 8.7$, $J_{4,5b} = 5.0$, $J_{4,5a} = 4.0$, H-4), 4.73 (d, 1H, $J_{2,3} = 5.1$, H-2), 4.46 (dd, 1H, H-3), 4.01 (dd, 1H, $^2J_{5a,5b} = 11.7$, H-5a), 3.93 (dd, 1H, H-5b), 3.08 (s, 3H, Me of Ms), 1.60 (s, 3H, $-\text{C}(\text{CH}_3)_2$), 1.42 (s, 3H, $-\text{C}(\text{CH}_3)_2$), 1.09 (s, 9H, $-\text{C}(\text{CH}_3)_3$). ^{13}C NMR (75.4 MHz, CDCl_3 , δ ppm) δ 135.7, 135.6, 132.1, 132.0, 130.4, 130.3, 128.3, 125.2 (C-aromat.), 116.3 (CN), 112.7 ($-\text{C}(\text{CH}_3)_2$), 80.4 (C-4), 77.0 (C-3), 65.6 (C-2), 63.9 (C-5), 38.6 (Me of Ms), 27.1 ($-\text{C}(\text{CH}_3)_2$), 27.0 ($-\text{C}(\text{CH}_3)_3$), 26.1 ($-\text{C}(\text{CH}_3)_2$), 19.3 ($-\text{C}(\text{CH}_3)_3$). LSIMS m/z 526 [60%, $(\text{M}+\text{Na})^+$]. HRLSIMS m/z found 526.1711, calc. for $\text{C}_{25}\text{H}_{33}\text{NO}_6\text{SSiNa}$: 526.1696.

(3*R*,4*S*,5*S*)-5-[(*O*-*tert*-Butyldiphenylsilyl)hydroxymethyl]-3,4-isopropylidene-2-methyl-1-pyrroline-3,4-diol (6)



To a solution of **18** (8.6 g, 17.1 mmol) in anhydrous toluene (70 mL), MeMgBr (7.0 mL, 21.0 mmol) was added dropwise. The mixture was stirred at $70\text{ }^\circ\text{C}$ for 7 h and then cooled to r.t. Diethyl ether (70 mL) and sat. aq. sol. of NH_4Cl (70 mL) were successively added and the resulting solution was extracted. The aqueous phase was extracted twice with CH_2Cl_2 and the organic layers were washed with brine, dried over Na_2SO_4 , filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (Toluene/acetone, 30:1) to give **6** (5.3 g, 12.5 mmol, 73%) as a yellow oil. $[\alpha]_D^{23}$ -46.4 (c 1.03, CH_2Cl_2). IR (ν cm^{-1}) 2930, 2857, 1650, 1107, 1075, 687. ^1H NMR (300 MHz, CDCl_3 , δ ppm, J Hz) δ 7.64-7.58 (m, 4H, H-aromat.), 7.44-7.36 (m, 6H, H-aromat.), 4.97 (d, 1H, $J_{4,3} = 5.1$, H-4), 4.63 (d, 1H, H-3), 4.22-4.18 (m, 1H, H-5), 3.92 (dd, 1H, $^2J_{1'a,1'b} = 10.2$, $J_{1'a,5} = 2.7$, H-1'a), 3.86 (dd, 1H, $J_{1'b,5} = 3.0$, H-1'b), 2.15 (s, 3H, Me), 1.37 (s, 6H, $-\text{C}(\text{CH}_3)_2$), 1.01 (s, 9H, $-\text{C}(\text{CH}_3)_3$). ^{13}C NMR (75.4 MHz, CDCl_3 , δ ppm) δ 174.9 (C-2), 135.8, 135.6, 133.4, 132.9, 130.0, 129.9, 127.92, 127.88 (C-aromat.), 111.6 ($-\text{C}(\text{CH}_3)_2$), 87.8 (C-4), 81.1 (C-3), 77.0 (C-5), 63.9 (C-1'), 27.1 ($-\text{C}(\text{CH}_3)_2$), 26.9 ($-\text{C}(\text{CH}_3)_3$), 26.1 ($-\text{C}(\text{CH}_3)_2$), 19.3 ($-\text{C}(\text{CH}_3)_3$), 17.0 (Me). LSIMS m/z 424 [34%, $(\text{M}+\text{H})^+$], 346 [75%, $(\text{M}-\text{C}_6\text{H}_5)^+$]. HRLSIMS m/z found 424.2309, calc. for $\text{C}_{25}\text{H}_{34}\text{NO}_3\text{Si}$: 424.2308.

4. Experimental details for the synthesis of azides a-u



- Azides **c**, **f**, **j** and **m** were purchased from Sigma-Aldrich.
- Azides **a**, **b**, **e**, **g-i**, **k**, **l** and **n** were synthesized in one pot from commercially available halides following the standard protocols previously reported.³ Nevertheless, they can be also obtained from commercial sources.
- Azides **q-u** have been previously prepared and characterized in our research group.⁴
- Azide **d**, although commercially available, was prepared following the same procedure than for azide **g**,^{3b} and characterized here for the first time.
- Azides **o** and **p** have been prepared here for the first time.

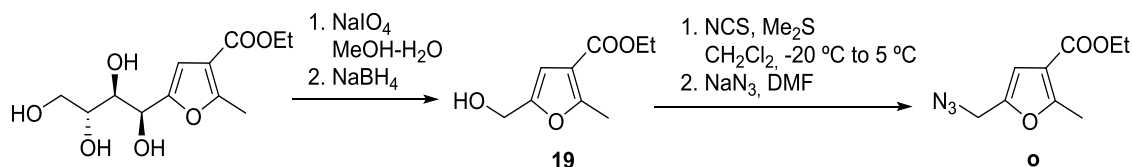
³ (a) For azide **a**, see: M. Lamani, P. Devadig, K. R. Prabhu, *Org. Biomol. Chem.*, 2012, **10**, 2753. (b) For azides **b**, **e** and **g**, see: T. Suzuki, Y. Ota, M. Ri, M. Bando, A. Gotoh, Y. Itoh, H. Tsumoto, P. R. Tatum, T. Mizukami, H. Nakagawa, S. Iida, R. Ueda, K. Shirahige, N. Miyata, *J. Med. Chem.*, 2012, **55**, 9562. (c) For azide **h**, see: E. Bellur, M. A. Yawer, I. Hussain, A. Riahi, O. Fatunsin, C. Fischer, P. Langer *Synthesis*, 2009, 227. (d) For azide **i**, see: J.-M. Kee, R. C. Oslund, D. H. Perlman, T. W. Muir *Nat. Chem. Biol.*, 2013, **9**, 416. (e) For azide **k**, see: N. J. Stanley, D. Pedersen, Sejer, Nielsen, B.; Kvist, T.; Mathiesen, J. M.; Braeuner-Osborne, H.; Taylor, D. K.; Abell, A. D. *Bioorg. Med. Chem. Lett.*, 2010, **20**, 7512. (f) For azide **l**, see: J. Chen, X.-G. Fu, L. Zhou, J.-T. Zhang, X.-L. Qi, X.-P. Cao *J. Org. Chem.*, 2009, **74**, 4149. (g) For azide **n**, see: L. Díaz, J. Casas, J. Bujons, A. Llebaria, A. Delgado, *J. Med. Chem.*, 2011, **54**, 2069.

⁴ (a) For azides **q** and **r**, see: (i) L. Molina, A. J. Moreno-Vargas, A. T. Carmona, I. Robina *Synlett*, 2006, 1327. (ii) L. Molina, E. Moreno-Clavijo, A. J. Moreno-Vargas, A. T. Carmona, I. Robina *Eur. J. Org. Chem.*, 2010, 3110. (b) For azides **s**, **t** and **u**, see: J. Ramos-Soriano, U. Niss, J. Angulo, M. Angulo, A. J. Moreno-Vargas, A. T. Carmona, S. Ohlson, I. Robina, *Chem. Eur. J.*, 2013, **19**, 17989.

4-(2-Azidoethyl)pyridine (d). IR (ν cm^{-1}) 2096 (N_3), 1603, 1413, 1268, 794. ^1H NMR (300 MHz, CDCl_3 , δ ppm, J Hz) δ 8.63-8.61 (m, 2H, H-aromat.), 7.26-7.23 (m, 2H, H-aromat.), 4.41 (s, 2H, $-\text{CH}_2\text{N}_3$). ^{13}C NMR (75.4 MHz, CDCl_3 , δ ppm) δ 150.4, 144.5, 122.5 (C-aromat.), 53.4 ($-\text{CH}_2\text{N}_3$). HRCIMS m/z found 134.0592, calc. for $\text{C}_6\text{H}_6\text{N}_4$: 134.0592.

General procedure for the synthesis of azides o and p

Ethyl 5-(azidomethyl)-2-methylfuran-3-carboxylate (o)



To a stirred solution of 3-ethoxycarbonyl-2-methyl-5-(*D*-arabinotetritol-1-yl)furan⁵ (2.91 g, 10.61 mmol) in MeOH (40 mL) cooled to 0 °C was added a solution of NaIO_4 (5.22 g, 24.40 mmol) in H_2O (30 mL), and the mixture was stirred for 1 h. Then, the solution was filtered, and NaBH_4 (804 mg, 21.22 mmol) was added to the filtrate. After 30 min, the solution was neutralized with citric acid (pH 7) and concentrated. The crude was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried (Na_2SO_4), filtered, and concentrated to give crude alcohol **19**. To a stirred solution of *N*-chlorosuccinimide (345 mg, 2.53 mmol) in anhydrous CH_2Cl_2 (8 mL) cooled to 0 °C, Me_2S (200 μL , 2.74 mmol) was added under N_2 . After 5 min, a sol. of alcohol **19** (358 mg, 1.94 mmol) in anhydrous CH_2Cl_2 (8 mL) at -20 °C was added under N_2 . The mixture was allowed to warm to 5 °C. After 1 h, the solvent was evaporated. The crude product chloromethyl derivative was dissolved in DMF, and NaN_3 (253 mg, 3.89 mmol) was added. The reaction mixture was stirred for 10 min. at r.t., then the solvent was evaporated, the resulting residue was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/cyclohexane, 1:8) to give pure azide **o** (342 mg, 1.63 mmol, 84%) as a colourless oil.

IR (ν cm^{-1}) 2981, 2932, 2094 (N_3), 1711 (C=O), 1222, 1079, 776. ^1H NMR (300 MHz, CDCl_3 , δ ppm, J Hz) δ 6.60 (s, 1H, H-4), 4.28 (q, 2H, $^3J_{\text{H,H}} = 7.2$, $-\text{CH}_2\text{CH}_3$), 4.23 (s, 2H, $-\text{CH}_2\text{N}_3$), 2.58 (s, 3H, Me), 1.34 (t, 3H, $-\text{CH}_2\text{CH}_3$). ^{13}C NMR (75.4 MHz, CDCl_3 , δ ppm) δ 163.8 (C=O), 160.1, 146.9, 114.5 (C-aromat.), 110.5 (C-4), 60.4 ($-\text{CH}_2\text{CH}_3$), 46.9 ($-\text{CH}_2\text{N}_3$), 14.5 ($-\text{CH}_2\text{CH}_3$), 14.0 (Me). HRCIMS m/z found 210.0874, calc. for $\text{C}_9\text{H}_{12}\text{O}_3\text{N}_3$: 210.0879.

⁵ (a) F. García-González *Adv. Carbohydr. Chem.*, 1956, **11**, 97. (b) G. Bartoli, J. G. Fernández-Bolaños, G. Di Antonio, G. Foglia, S. Giuli, R. Gunnella, M. Mancinelli, E. Marcantoni, M. Paoletti *J. Org. Chem.*, 2007, **72**, 6029.

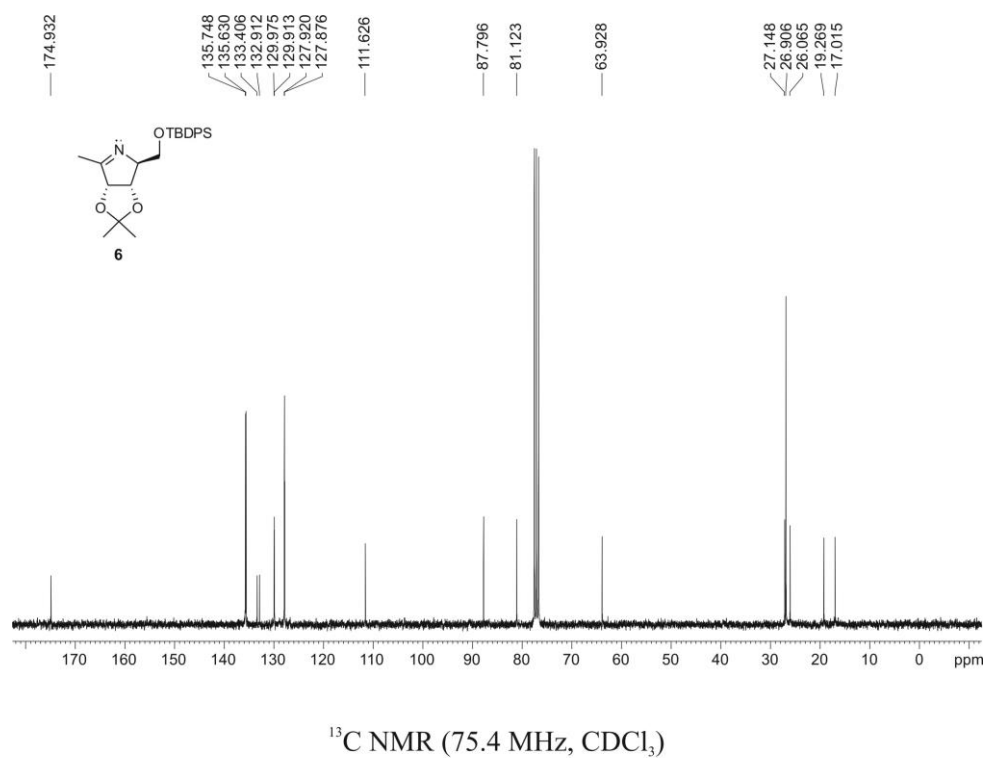
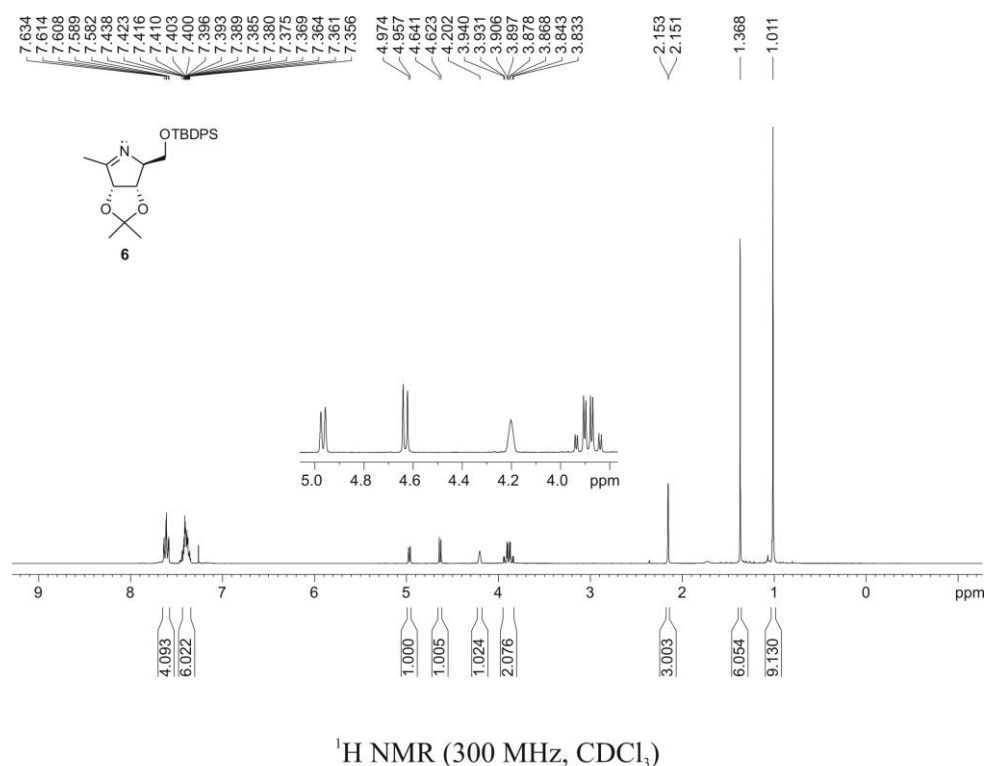
Benzyl 5-(azidomethyl)-2-methylfuran-3-carboxylate (p)

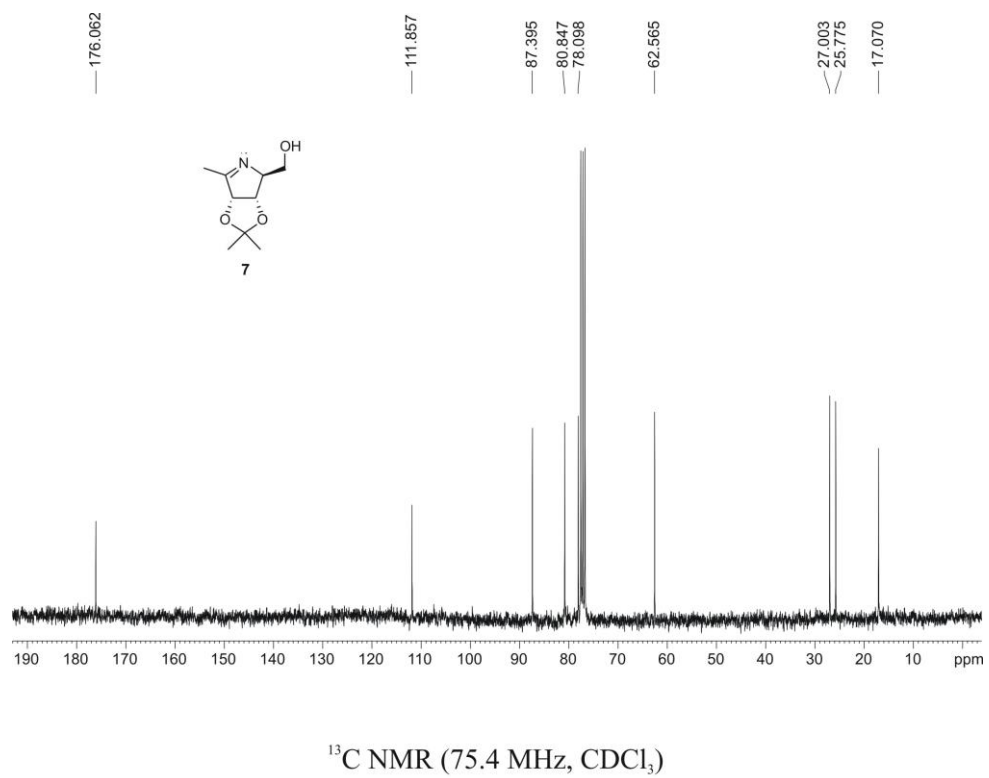
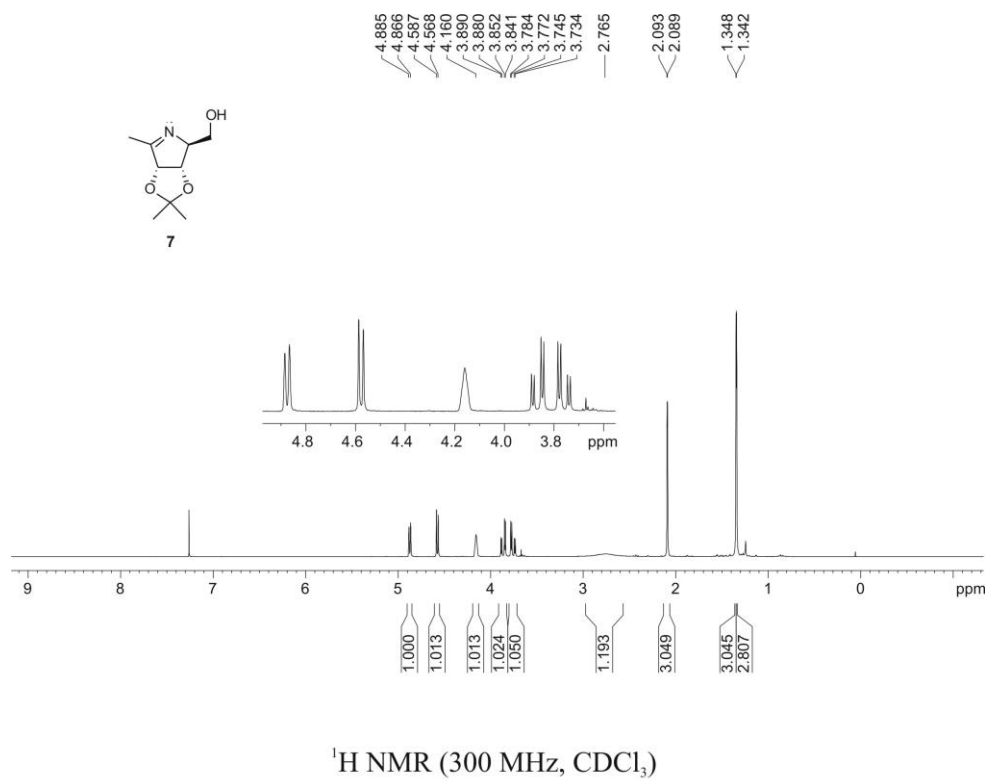
Azide **p** was synthesized as described for azide **o**, except that pure 3-benzyloxycarbonyl-2-methyl-5-(*D*-arabinotetritol-1-yl)furan⁶ was used as starting material. Azide **p** was obtained in 90% as a yellow oil.

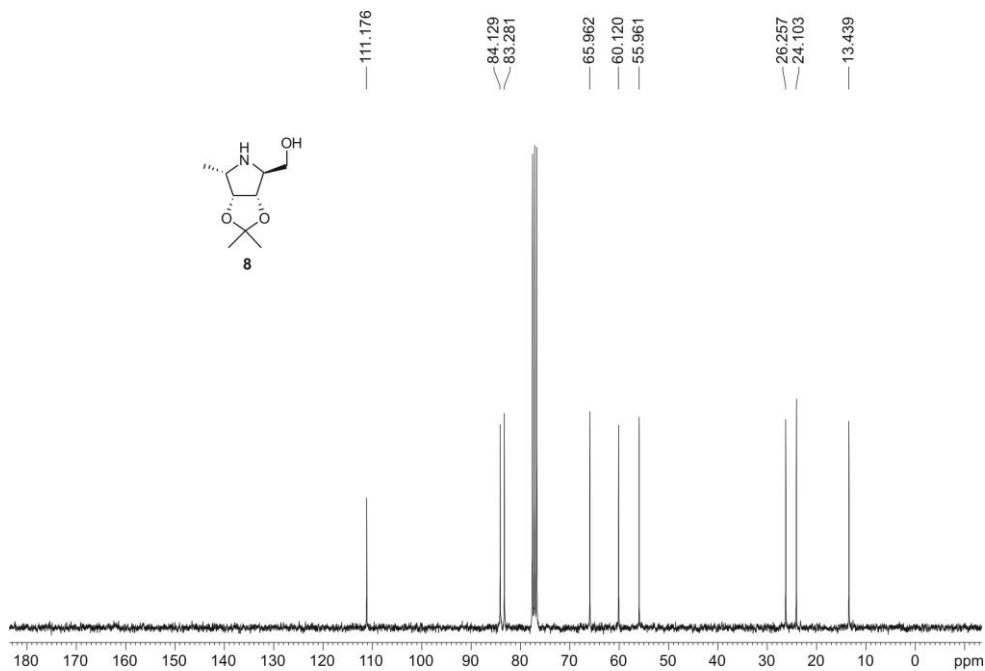
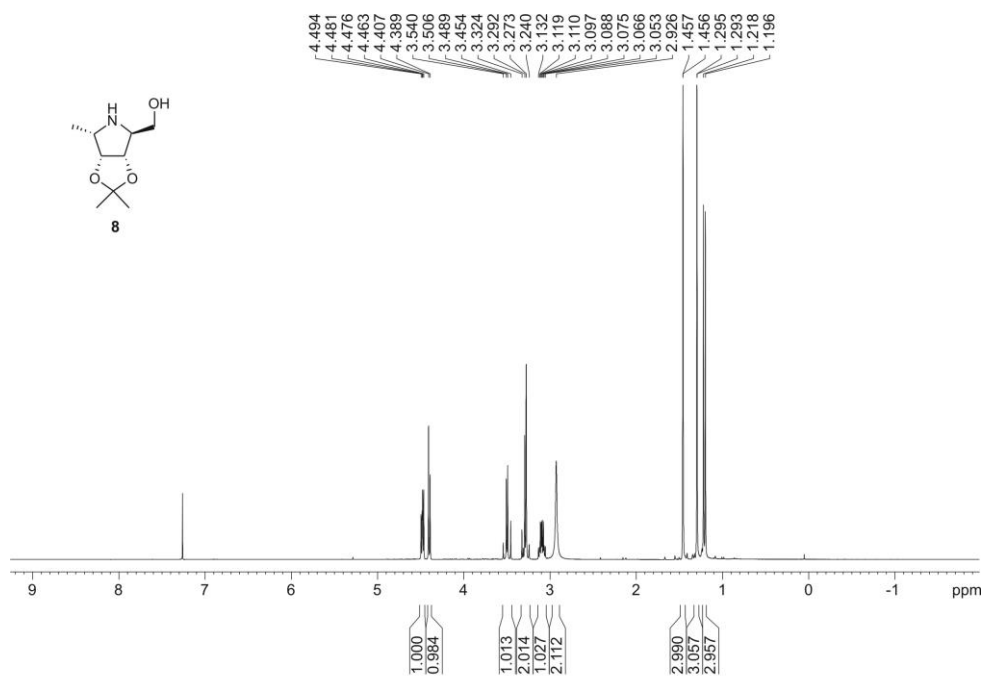
IR (ν cm^{-1}) 2952, 2095 (N_3), 1713 (C=O), 1214, 1072, 697. ^1H NMR (300 MHz, CDCl_3 , δ ppm, *J* Hz) δ 7.39-6.63 (m, 5H, H-aromat.), 6.63 (s, 1H, H-4), 5.28 (s, 2H, $-\text{CH}_2\text{Ph}$), 4.23 (s, 2H, $-\text{CH}_2\text{N}_3$), 2.59 (s, 3H, Me). ^{13}C NMR (75.4 MHz, CDCl_3 , δ ppm) δ 163.6 (C=O), 160.5, 147.1, 136.2, 128.7, 128.4, 128.3, 114.3 (C-aromat.), 110.5 (C-4), 66.2 ($-\text{CH}_2\text{Ph}$), 46.9 ($-\text{CH}_2\text{N}_3$), 14.1 (Me). HRCIMS *m/z* found 271.0947, calc. for $\text{C}_{14}\text{H}_{13}\text{O}_3\text{N}_3$: 271.0957.

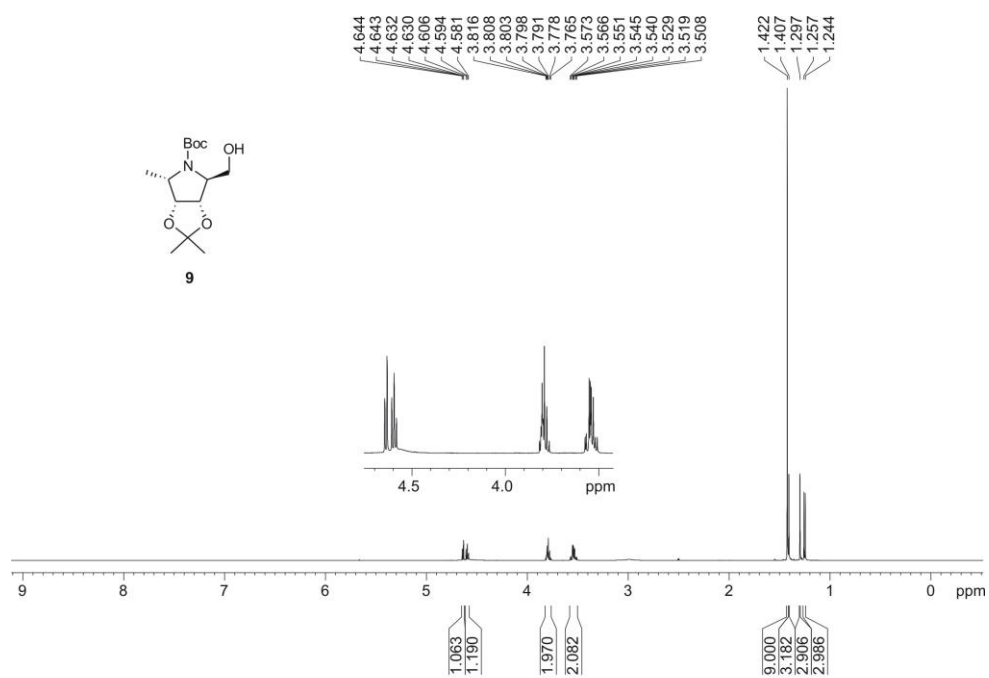
⁶ G. Coste, T. Horlacher, L. Molina, A. J. Moreno-Vargas, A. T. Carmona, I. Robina, P. H. Seeberger, S. Gerber-Lemaire, *Synthesis*, 2011, **11**, 1759.

5. Copies of ^1H and ^{13}C NMR Spectra

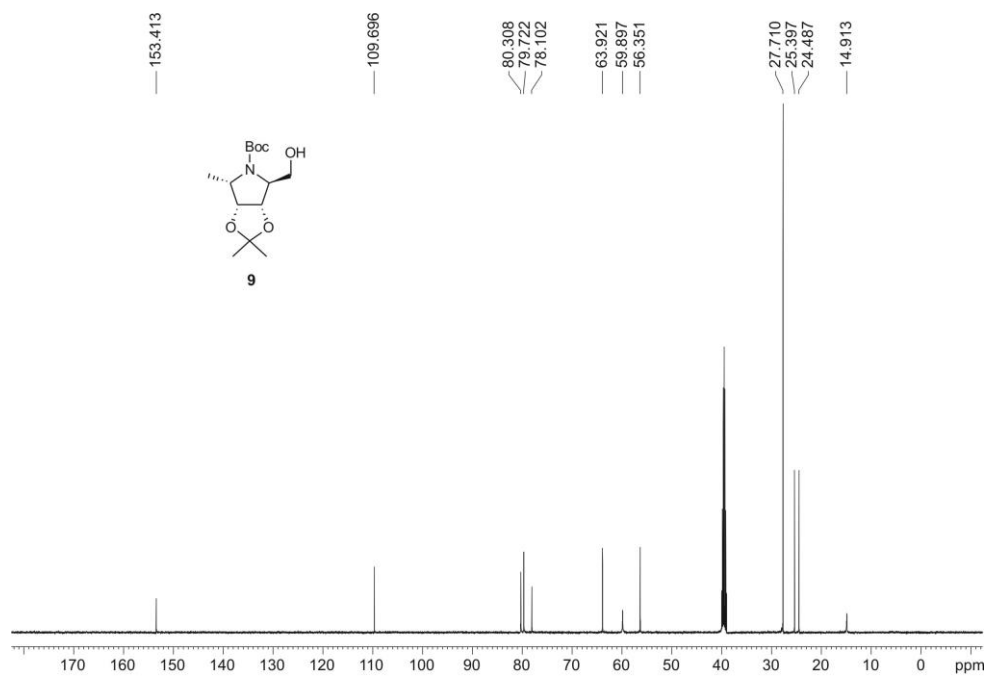




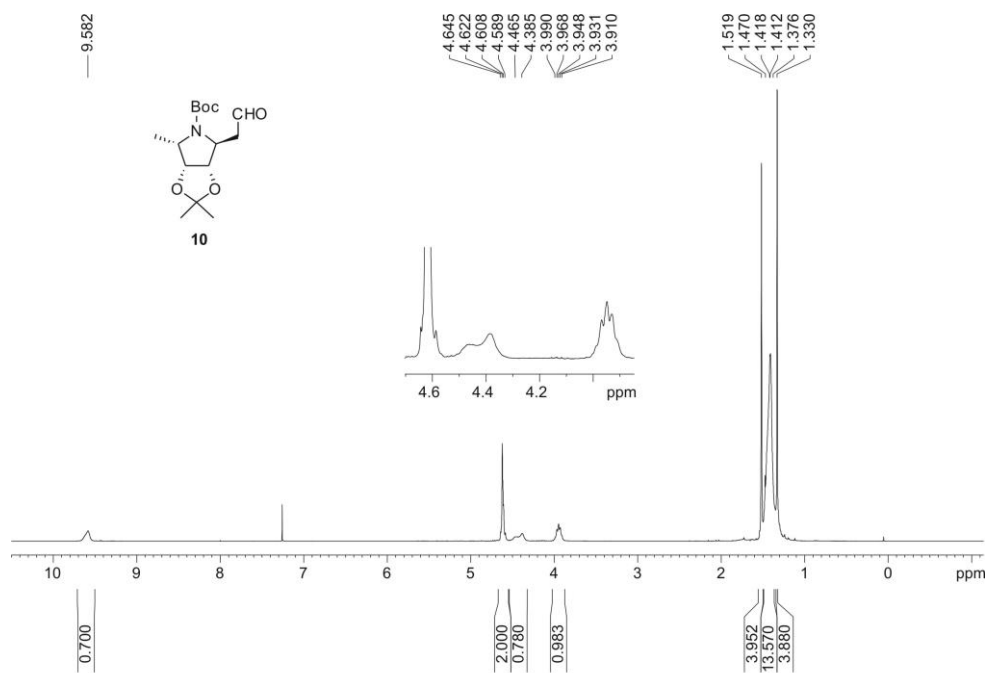




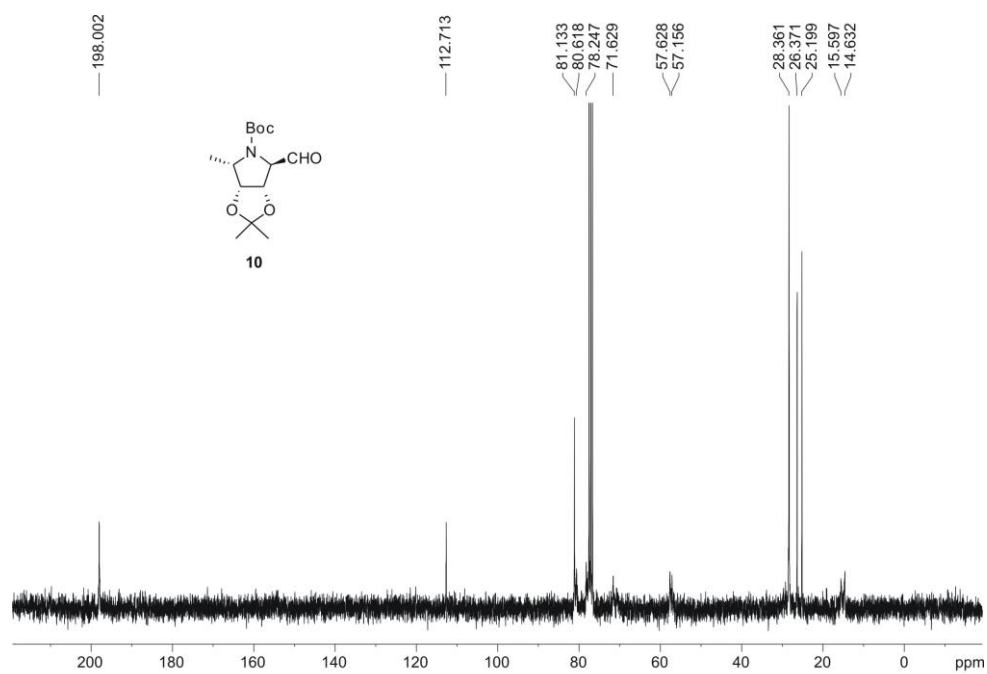
$^1\text{H NMR}$ (500 MHz, DMSO-d_6 , 363K)



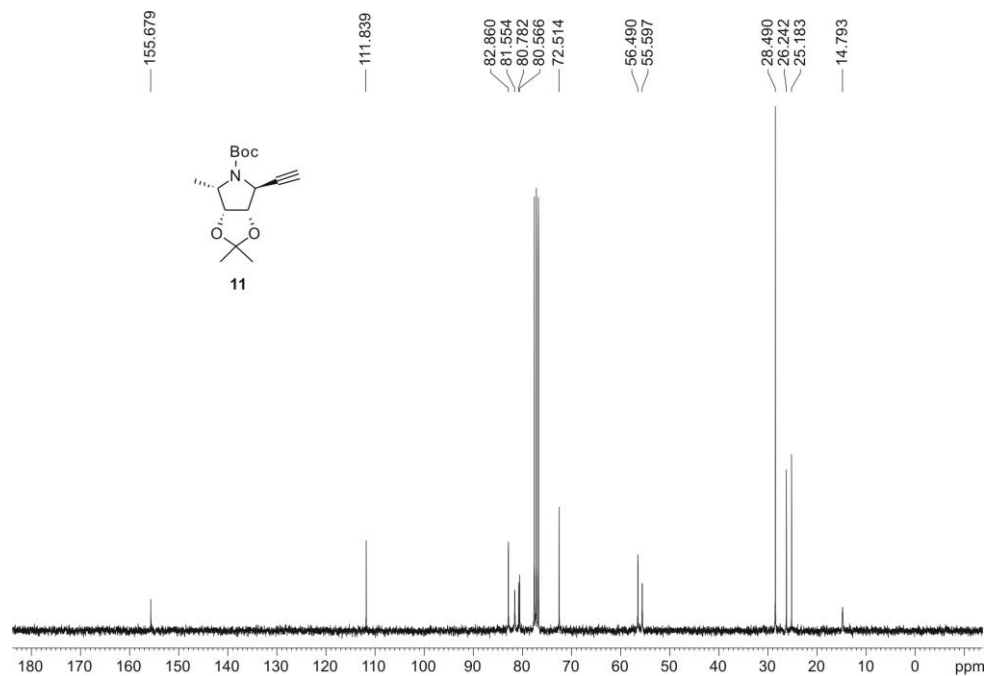
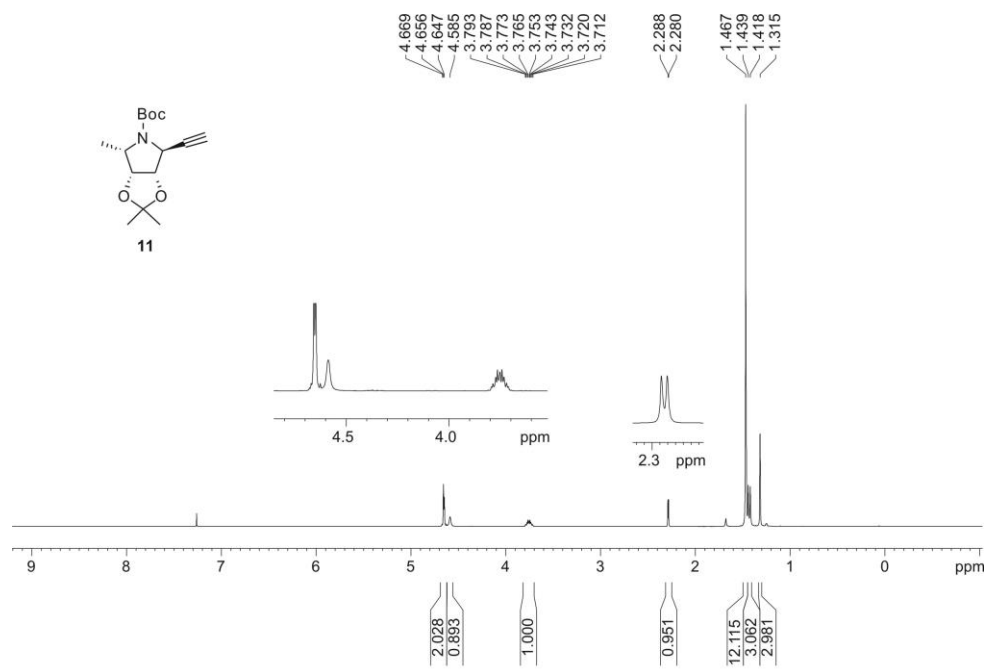
$^{13}\text{C NMR}$ (125.7 MHz, DMSO-d_6 , 363K)

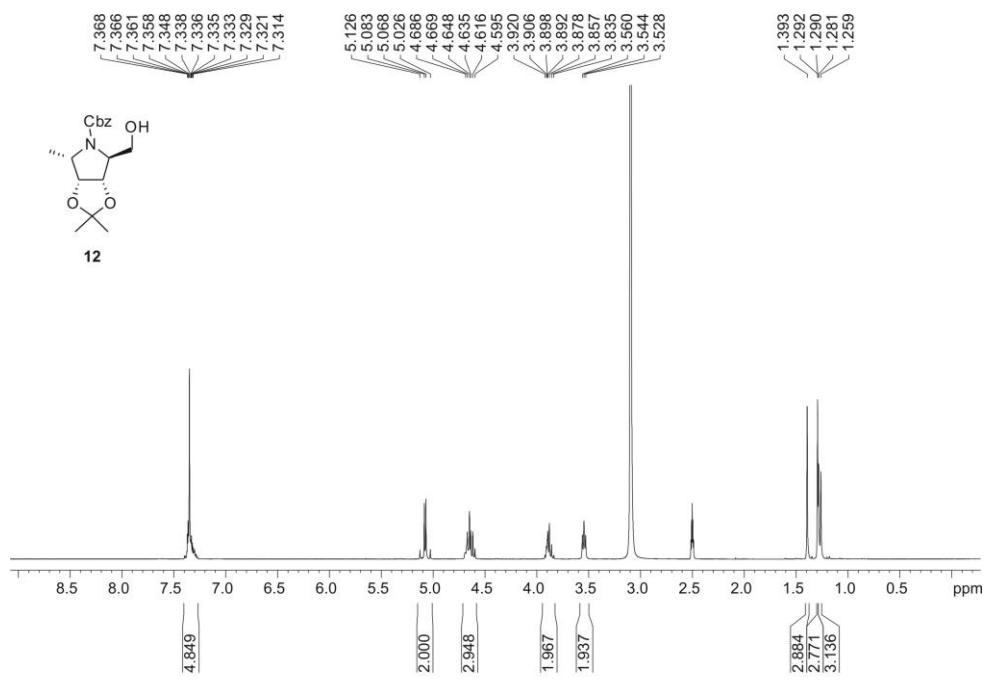


$^1\text{H NMR}$ (300 MHz, CDCl_3)

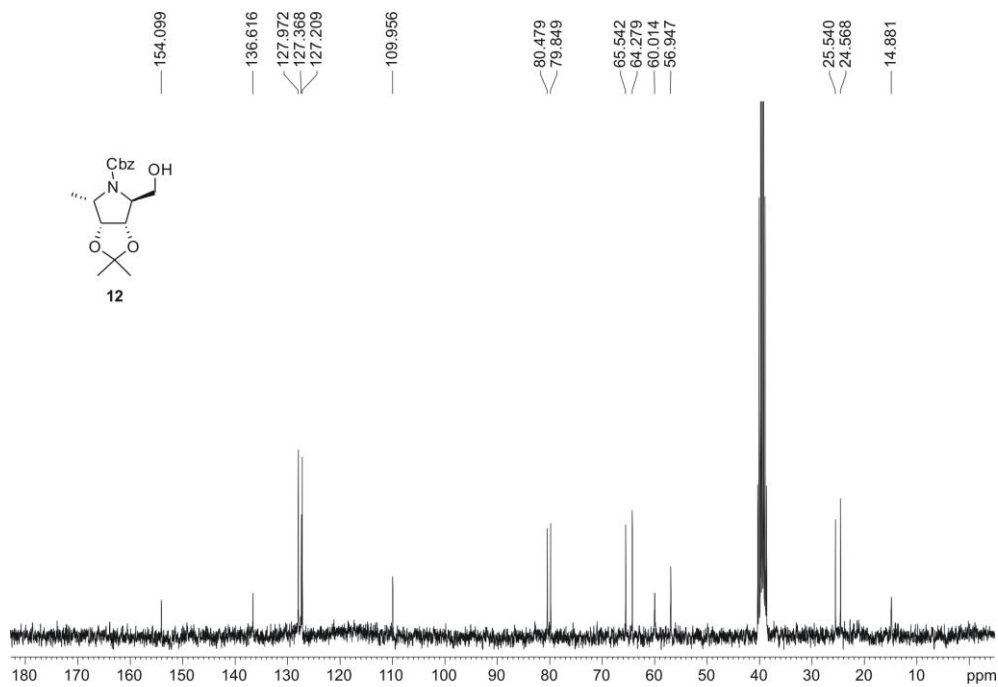


$^{13}\text{C NMR}$ (75.4 MHz, CDCl_3)

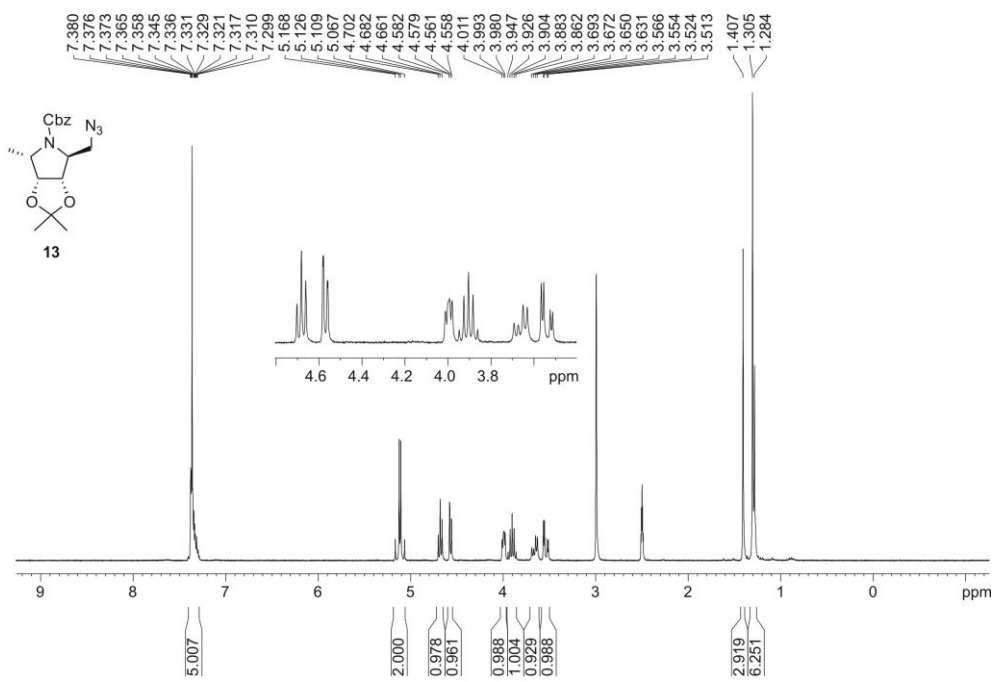




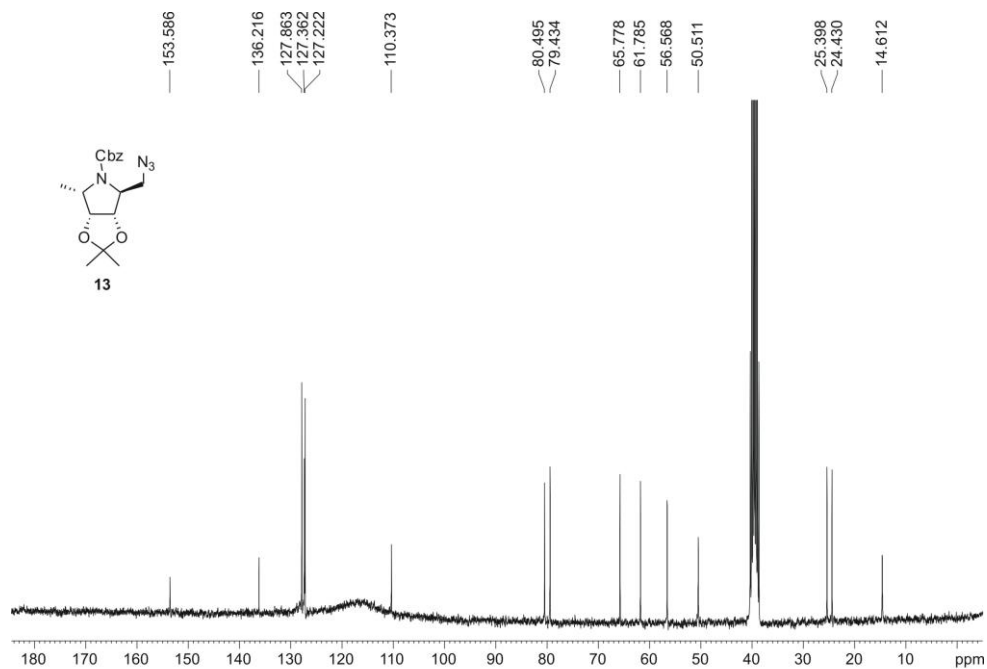
¹H NMR (300 MHz, DMSO-d₆, 363K)



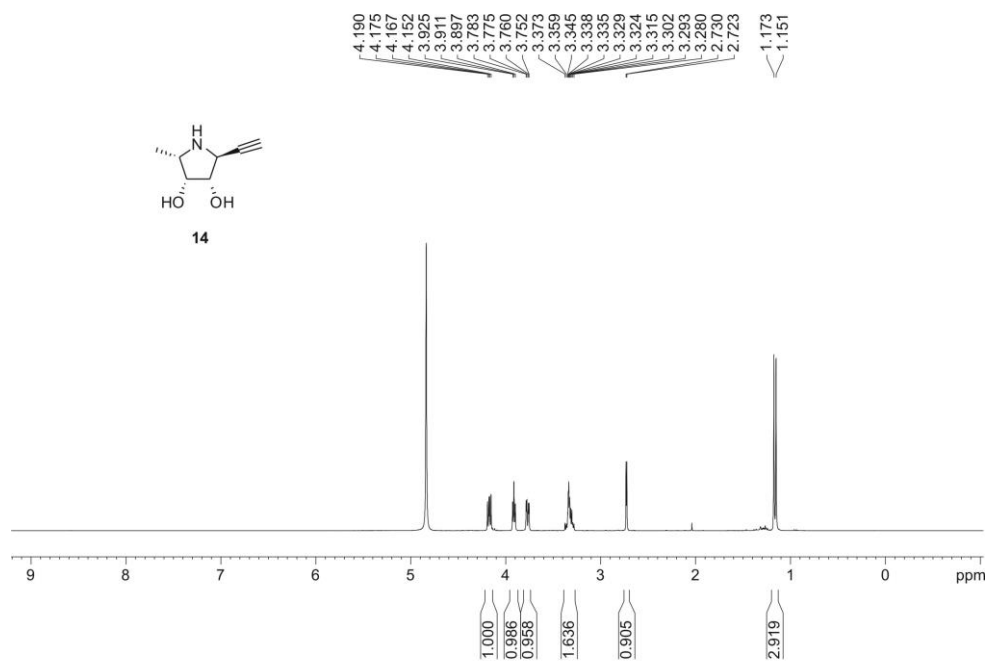
¹³C NMR (75.4 MHz, DMSO-d₆, 363K)



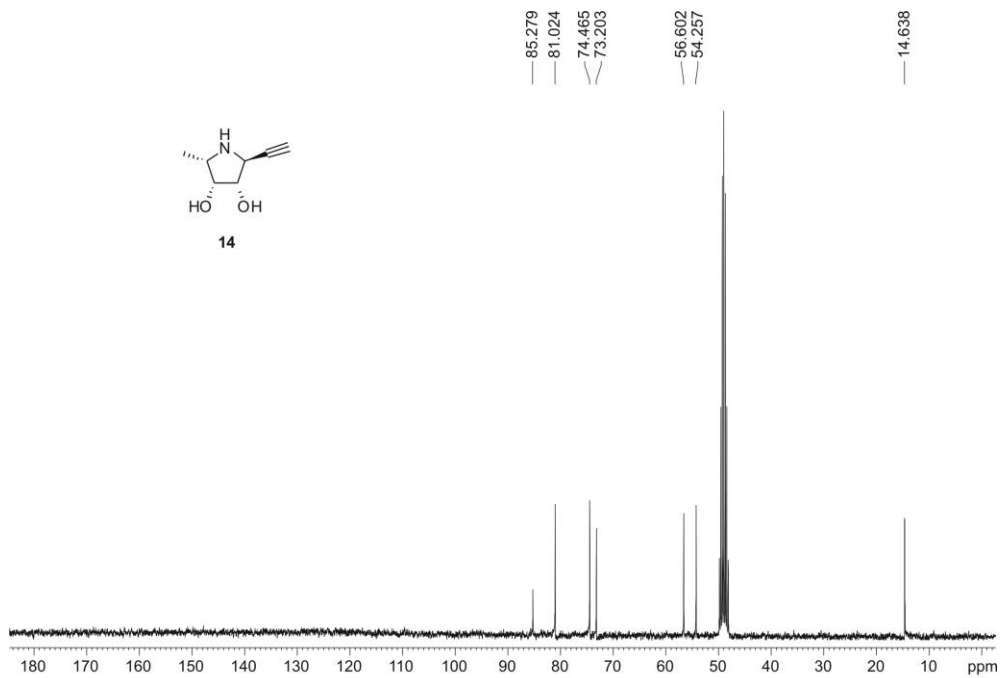
¹H NMR (300 MHz, DMSO-d₆, 363K)



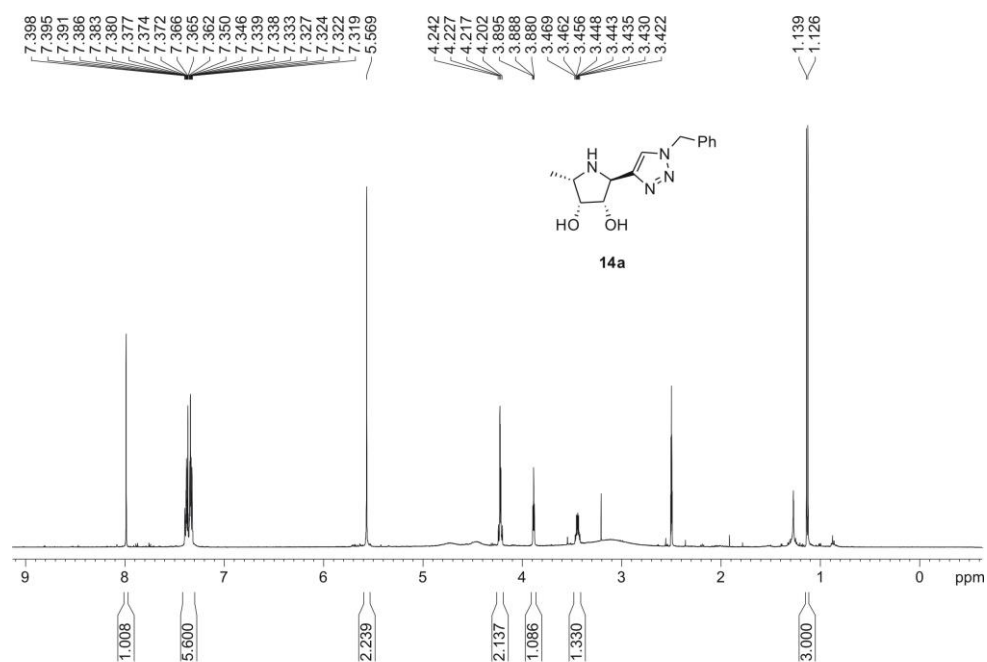
¹³C NMR (75.4 MHz, DMSO-d₆, 363K)



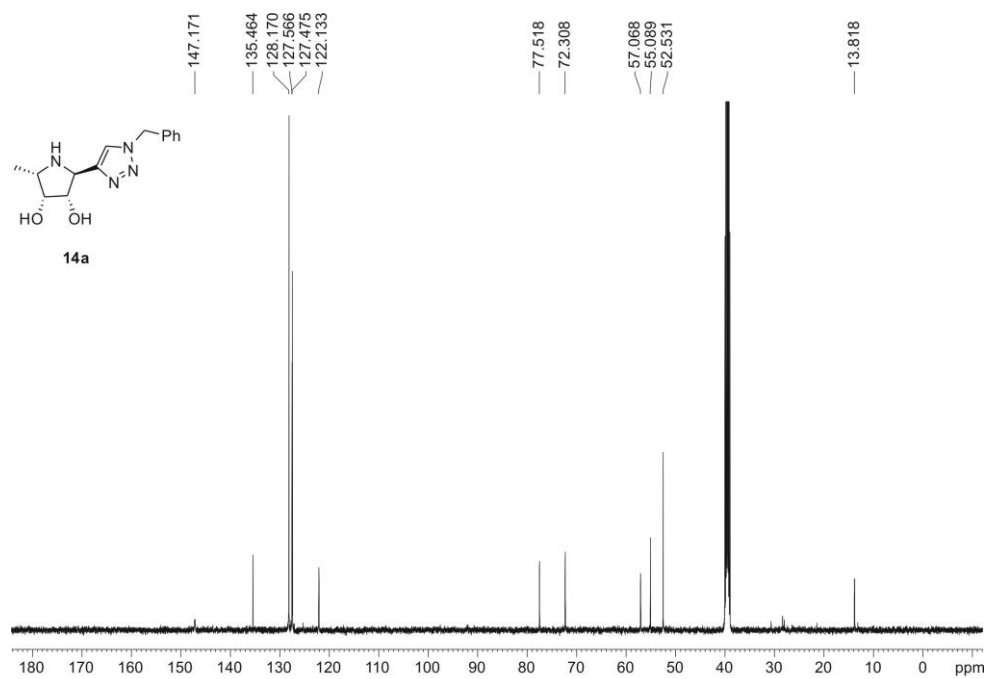
^1H NMR (300 MHz, MeOD)



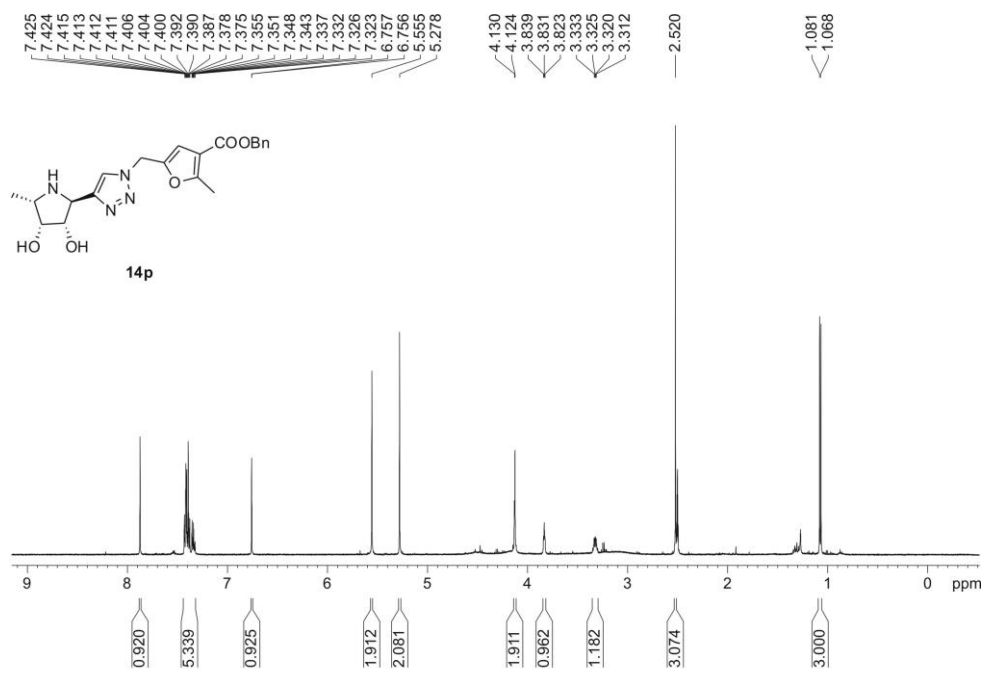
^{13}C NMR (75.4 MHz, MeOD)



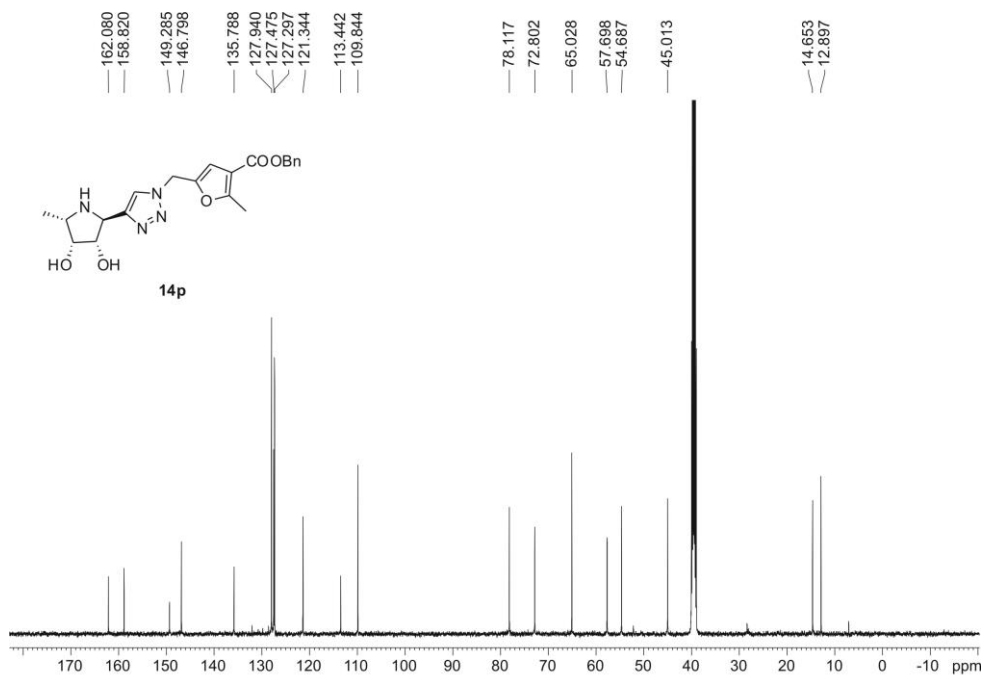
¹H NMR (500 MHz, DMSO-d₆, 363K)



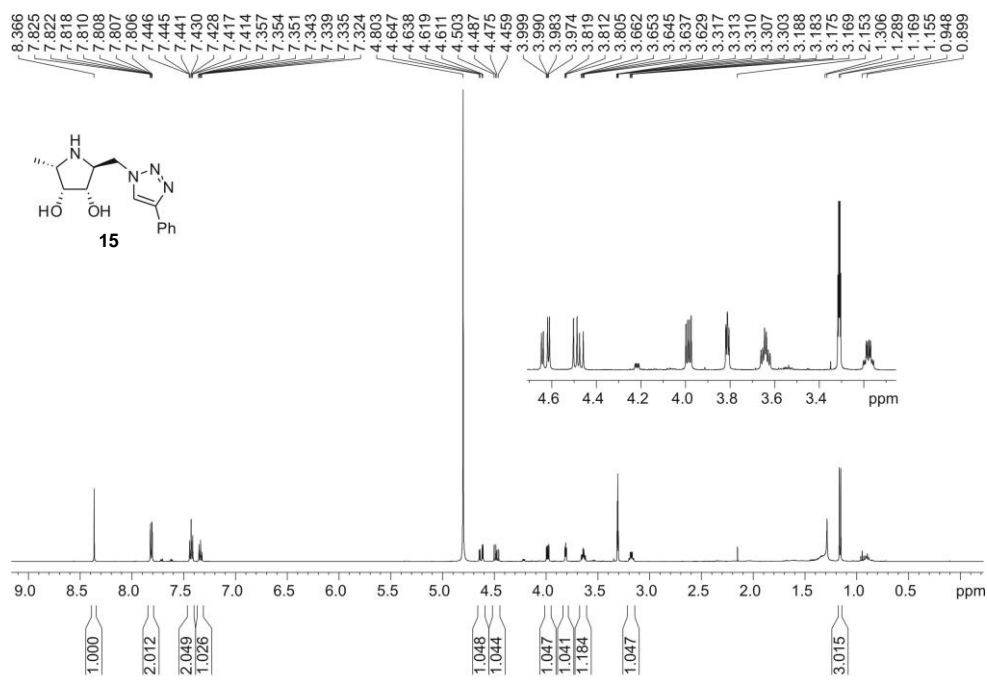
¹³C NMR (125.7 MHz, DMSO-d₆, 363K)



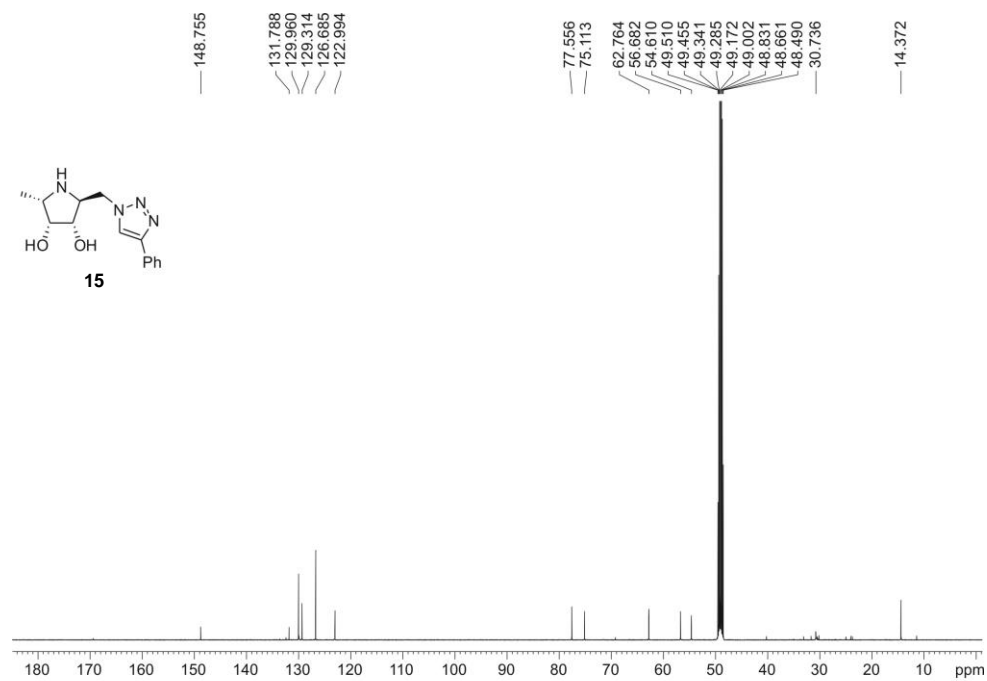
¹H NMR (500 MHz, DMSO-d₆, 363K)



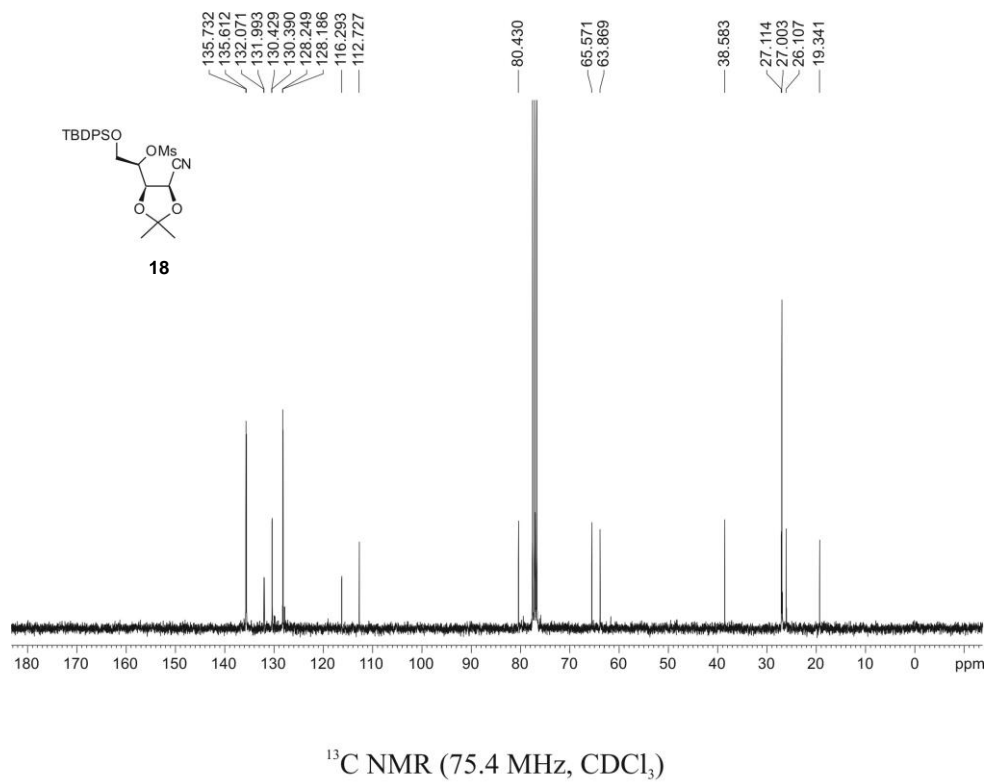
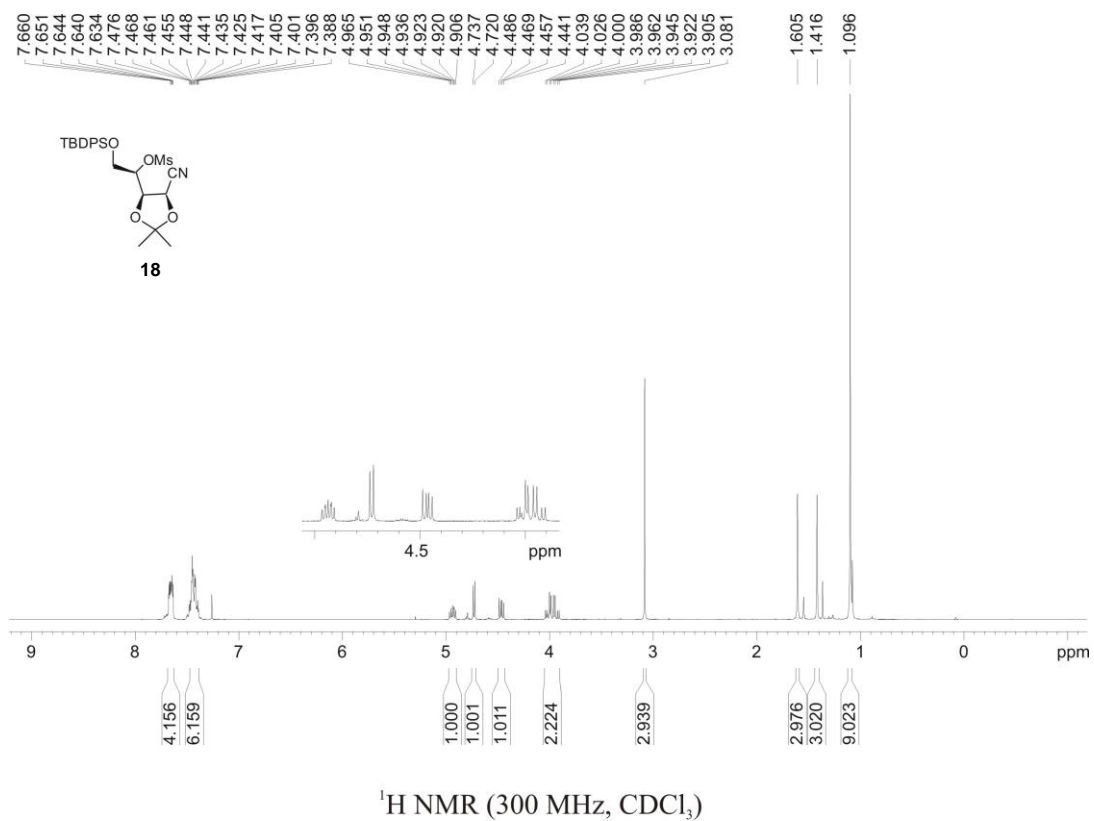
¹³C NMR (125.7 MHz, DMSO-d₆, 363K)

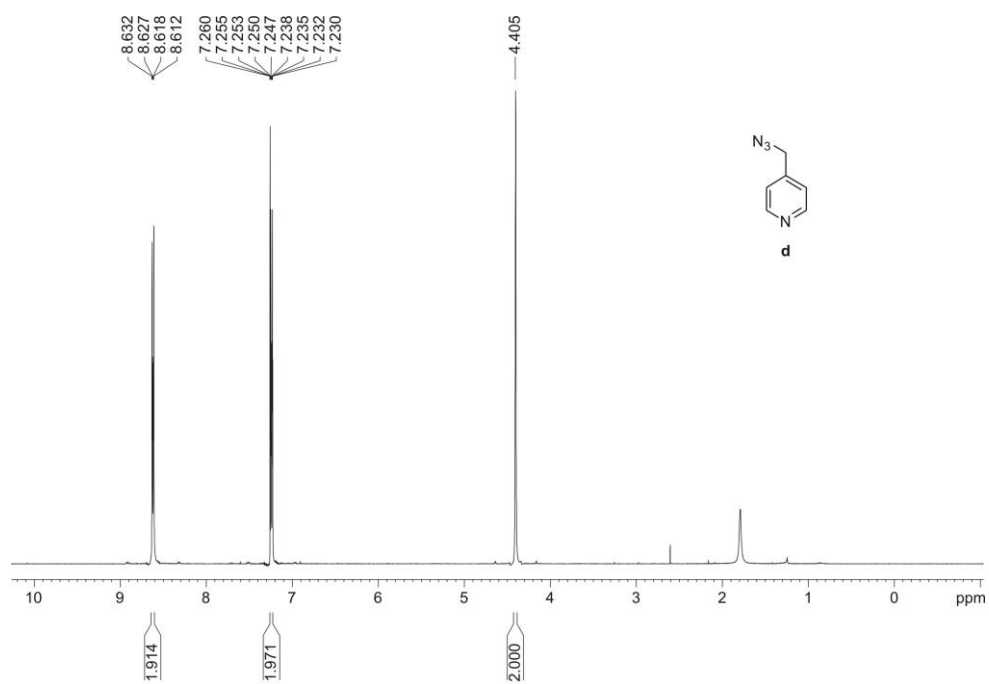


¹H NMR (500 MHz, MeOD)

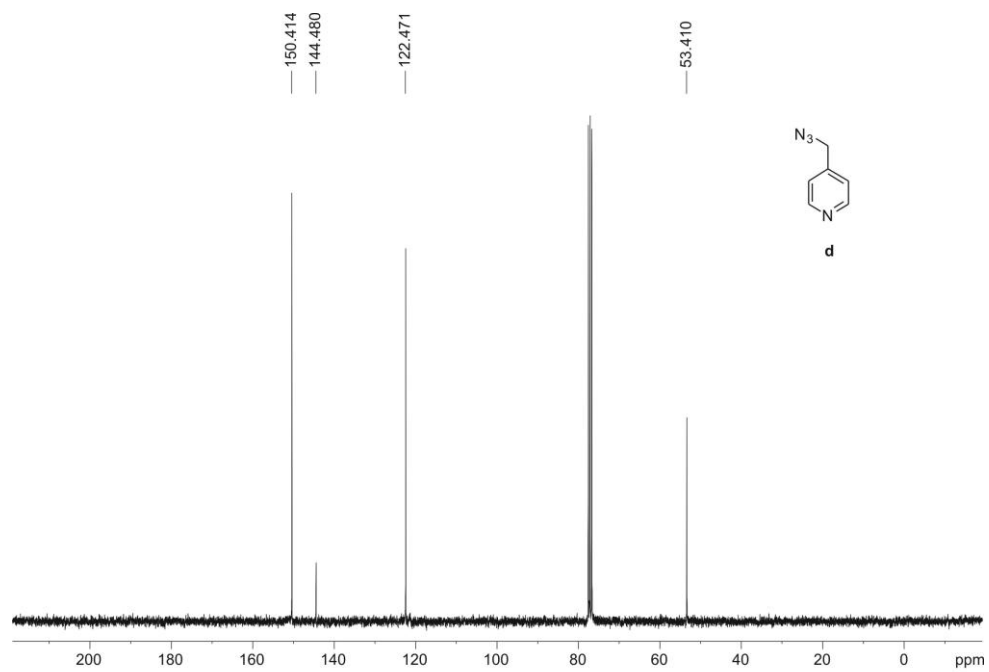


¹³C NMR (125.7 MHz, MeOD)

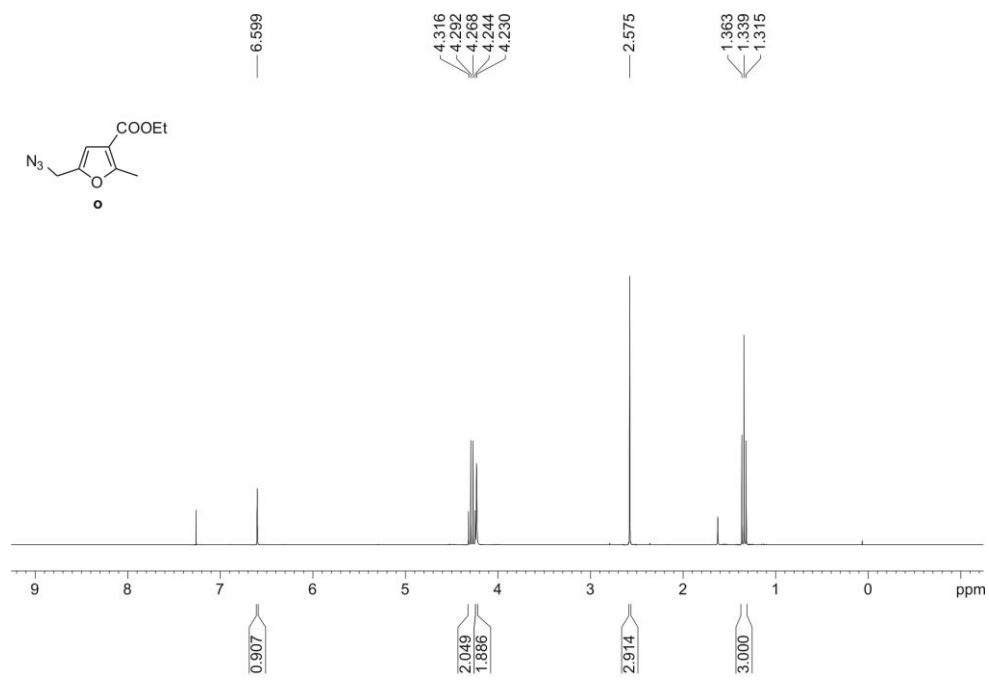




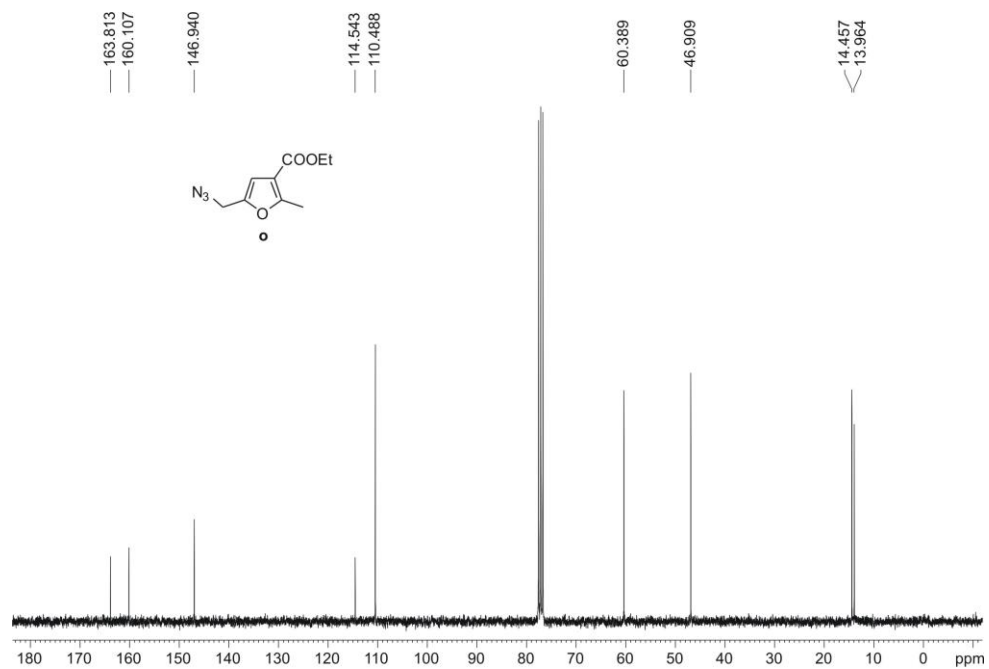
^1H NMR (300 MHz, CDCl_3)



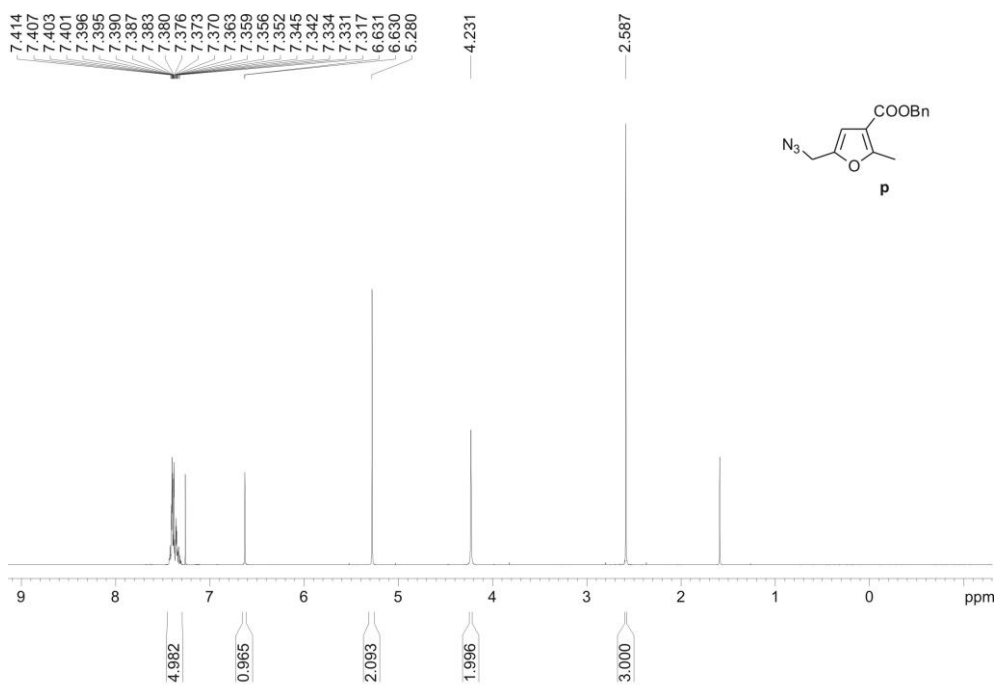
^{13}C NMR (75.4 MHz, CDCl_3)



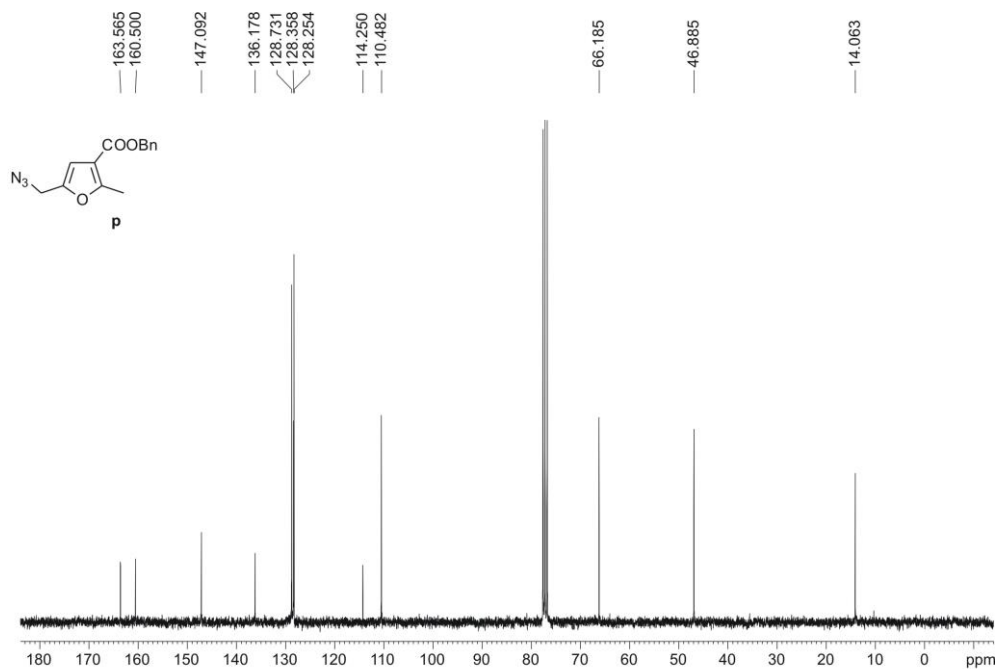
^1H NMR (300 MHz, CDCl_3)



^{13}C NMR (75.4 MHz, CDCl_3)



^1H NMR (300 MHz, CDCl_3)



^{13}C NMR (75.4 MHz, CDCl_3)