Supplementary Material

Rational design and regioselective synthesis of conformationally restricted furan-derived ligands as potential anti-malarial agents

Marius K. Mutorwa,[a] Iviwe Nokalipa,[a] Delia C. Tanner,[b] Gregory L. Blatch,[b,c] Kevin A. Lobb,[a,c] Rosalyn Klein, [a,c] and Perry T. Kaye[a,c] *

[a]Department of Chemistry, [b]Biomedical Biotechnology Research Unit and Department of Biochemistry and Microbiology and [c]Centre for Chemico- and Biomedicinal Research, Rhodes University, Grahamstown, 6140, South Africa

E-mail: P.Kaye@ru.ac.za

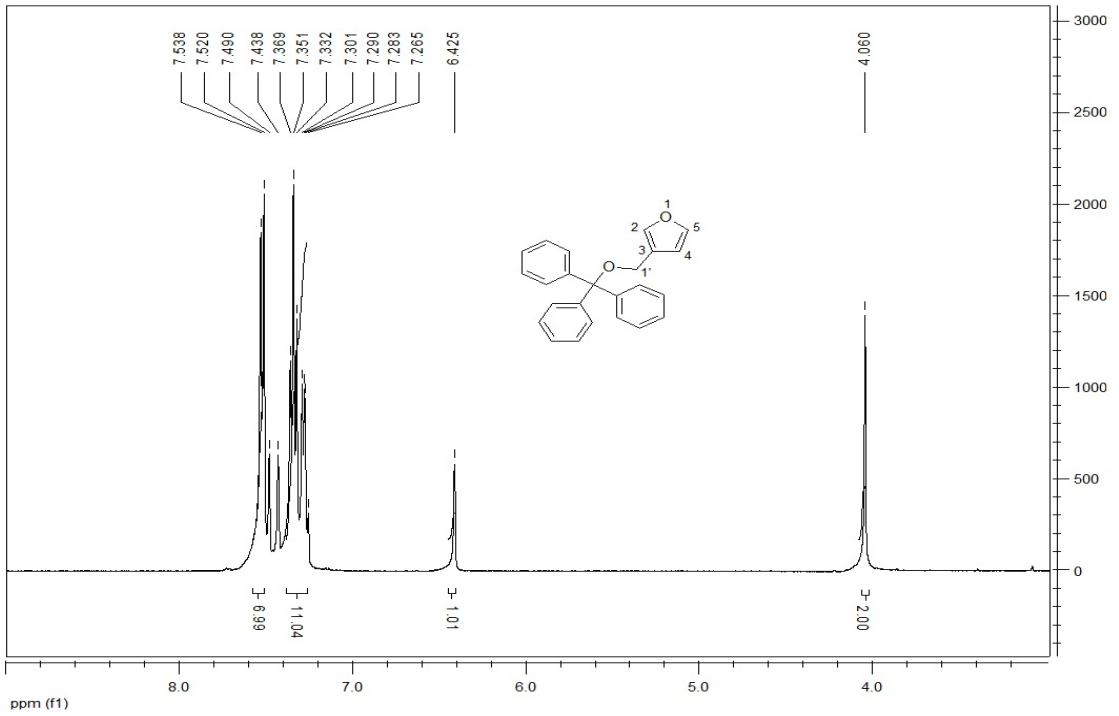
Table of Contents

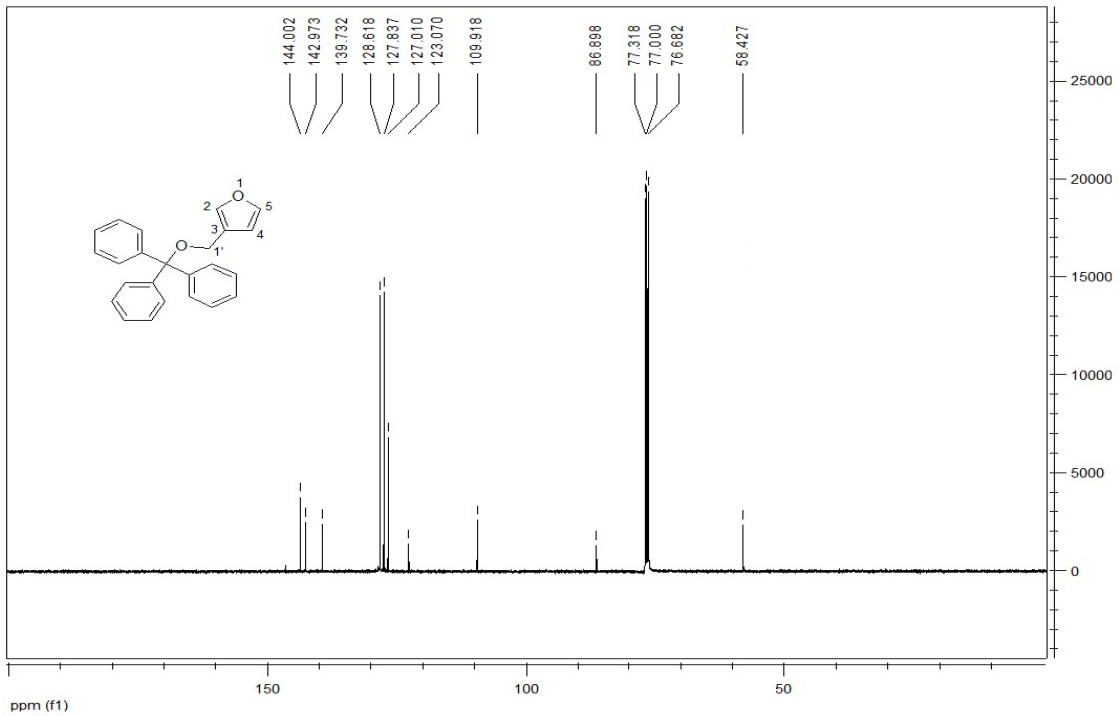
I. NMR Spectra S2
II. *In Silico* Modelling Data S24
III. Saturation Transfer Difference (STD) NMR binding protocol S29

 ©AUTHOR(S)

I. NMR Spectra

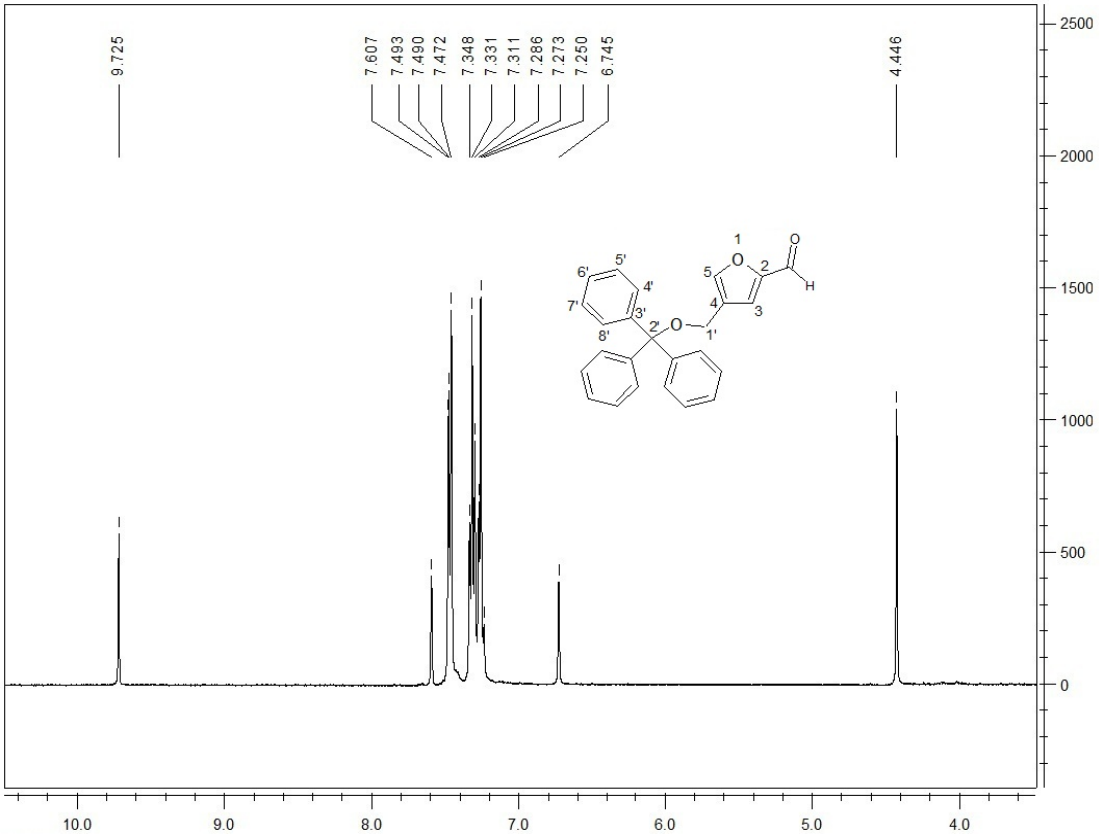
3-[(Trityloxy)methyl]furan 5

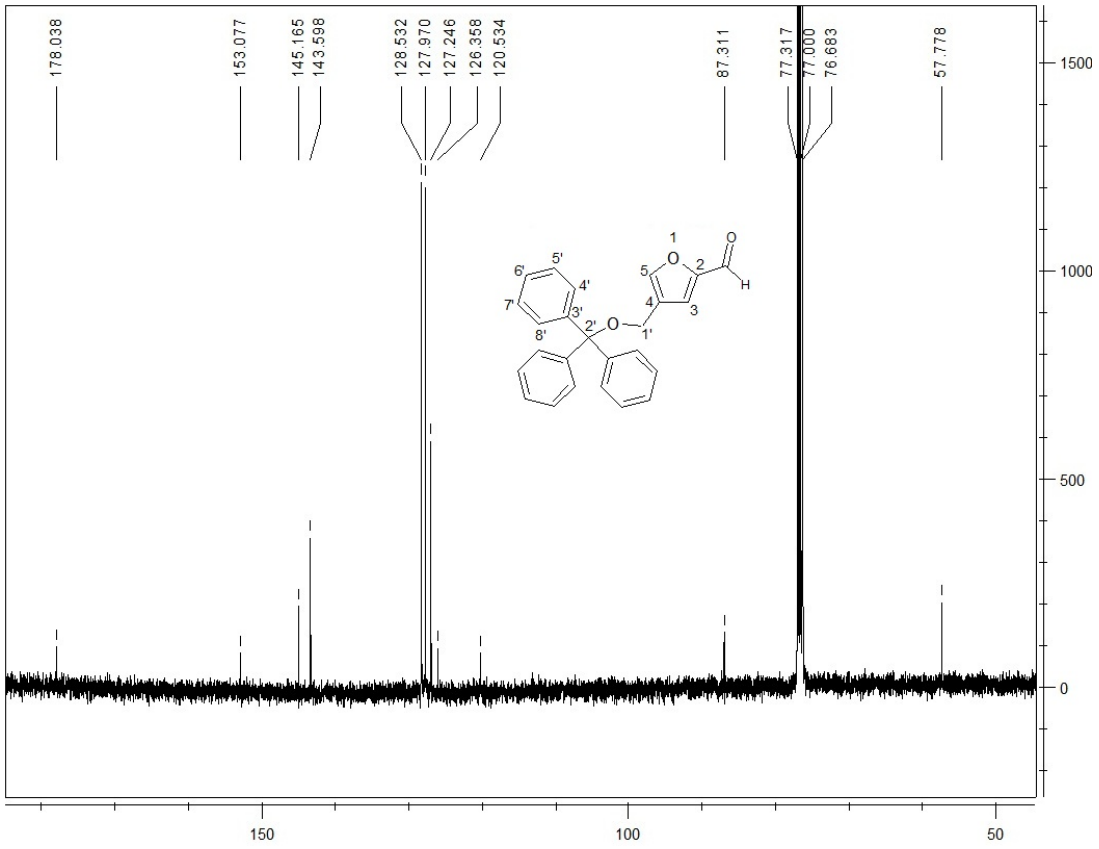




©AUTHOR(S)

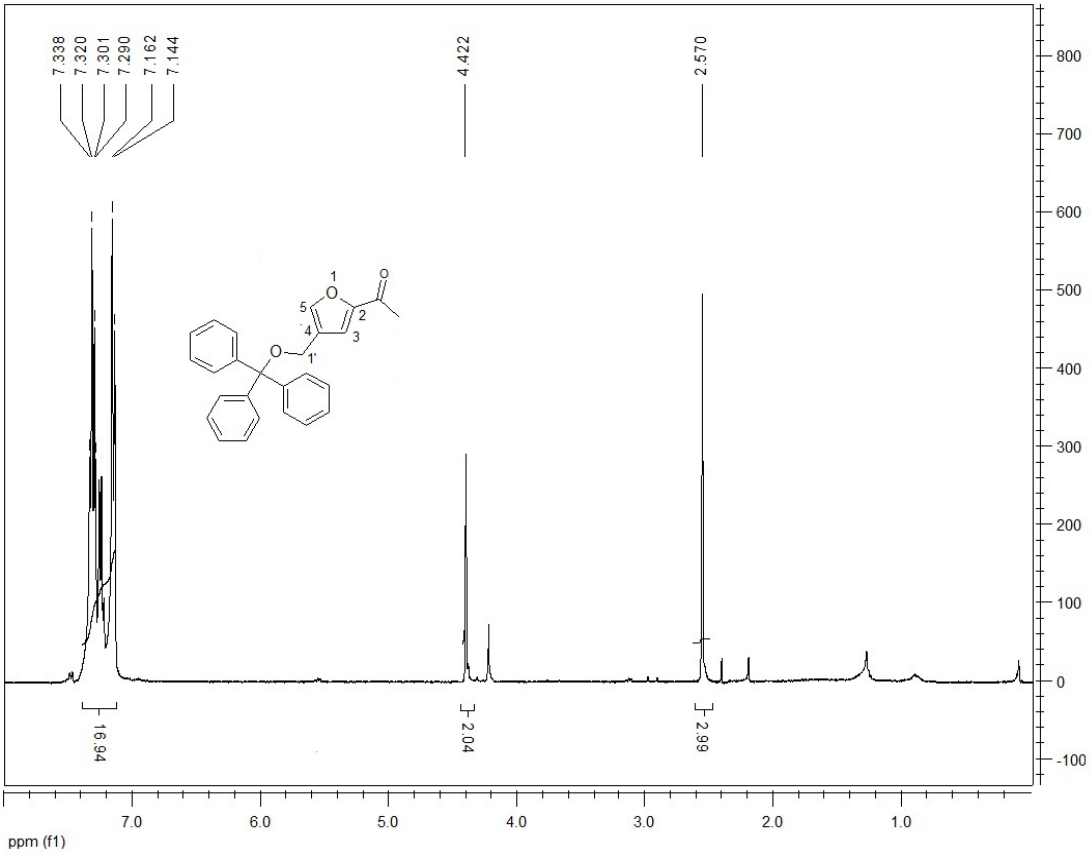
4-[(trityloxy)methyl]furan-2-carbaldehyde 6a

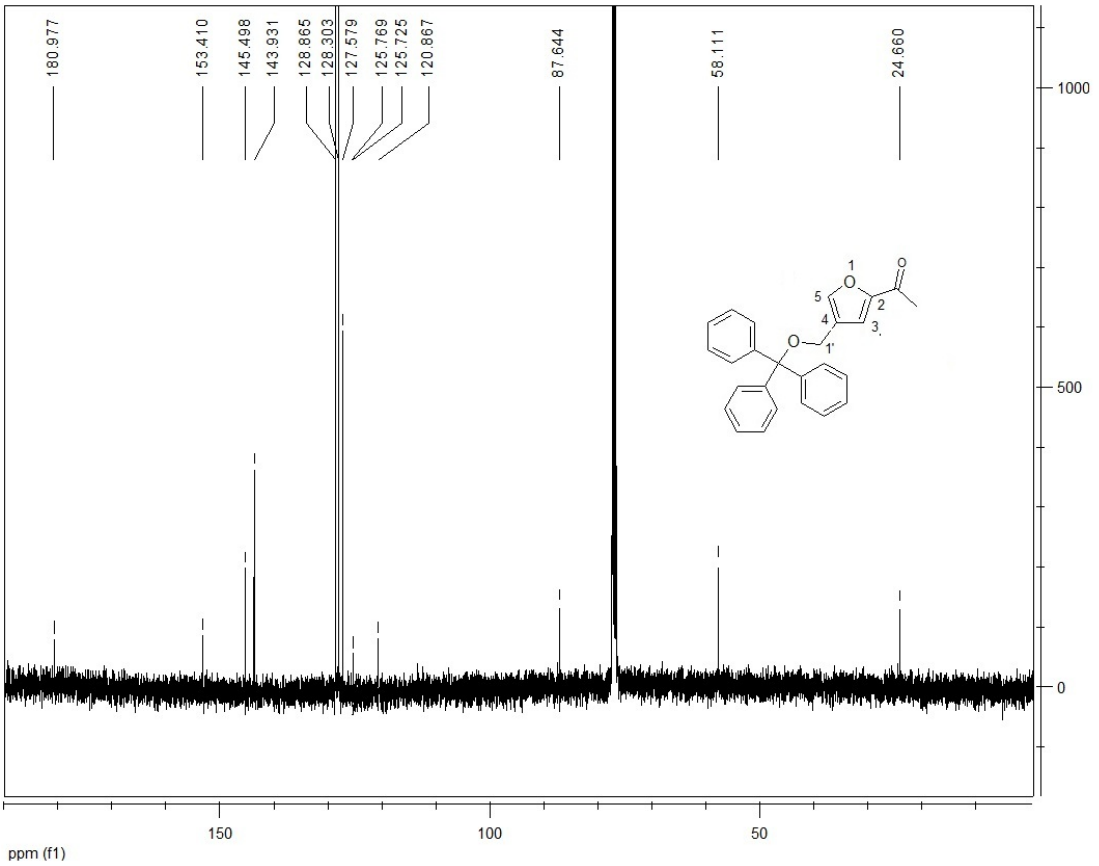




 ©AUTHOR(S)

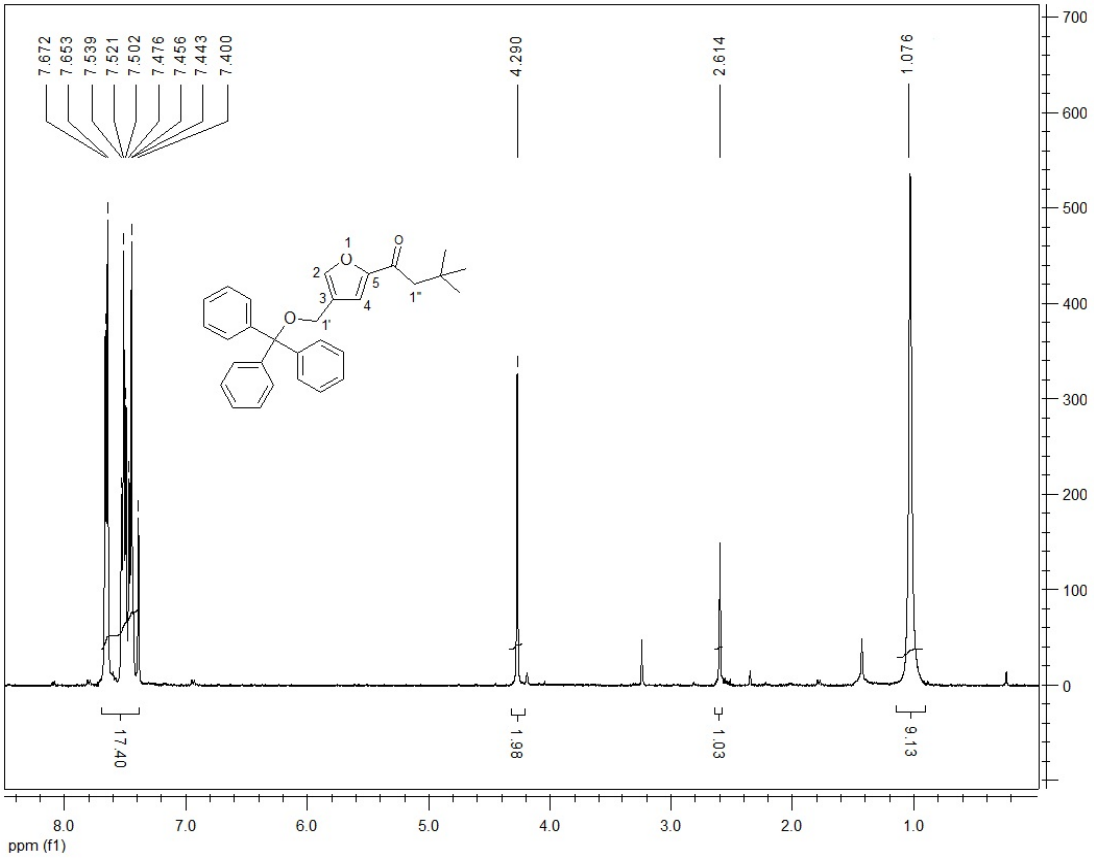
2-Acetyl-4-[(trityloxy)methyl]furan 6b

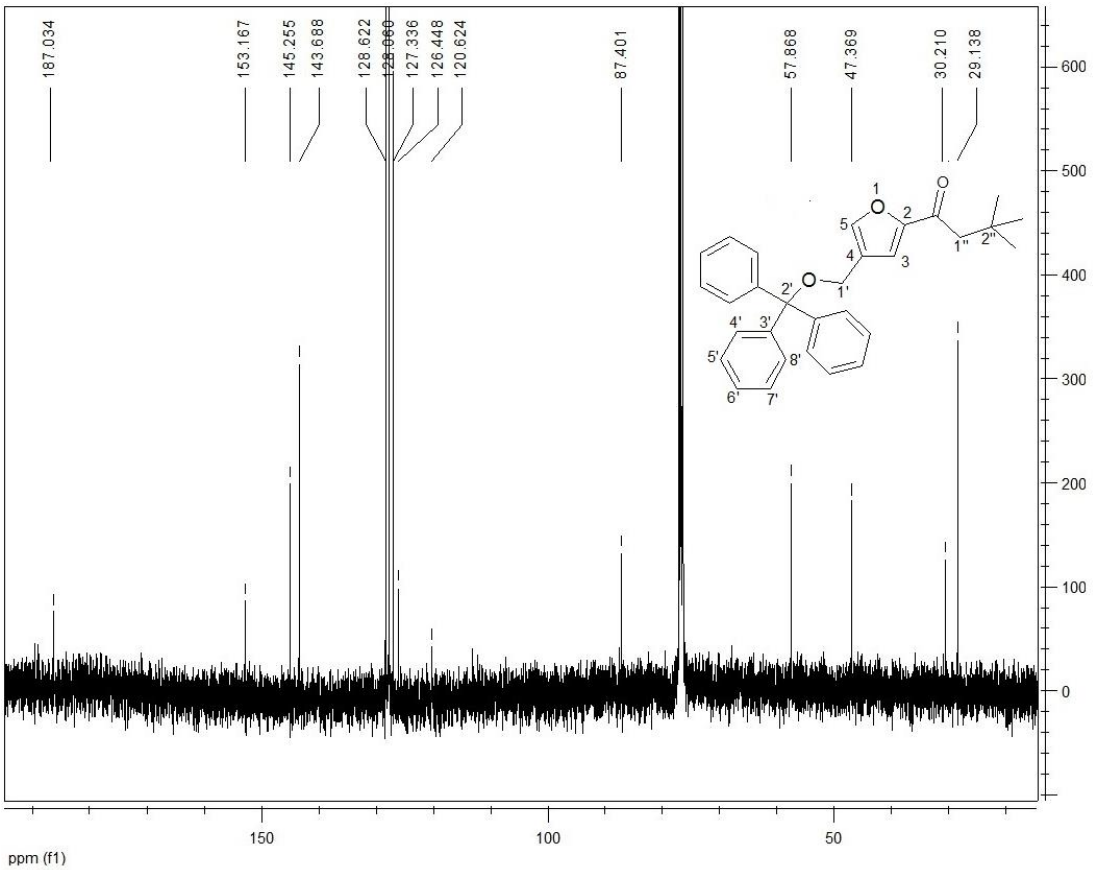




 ©AUTHOR(S)

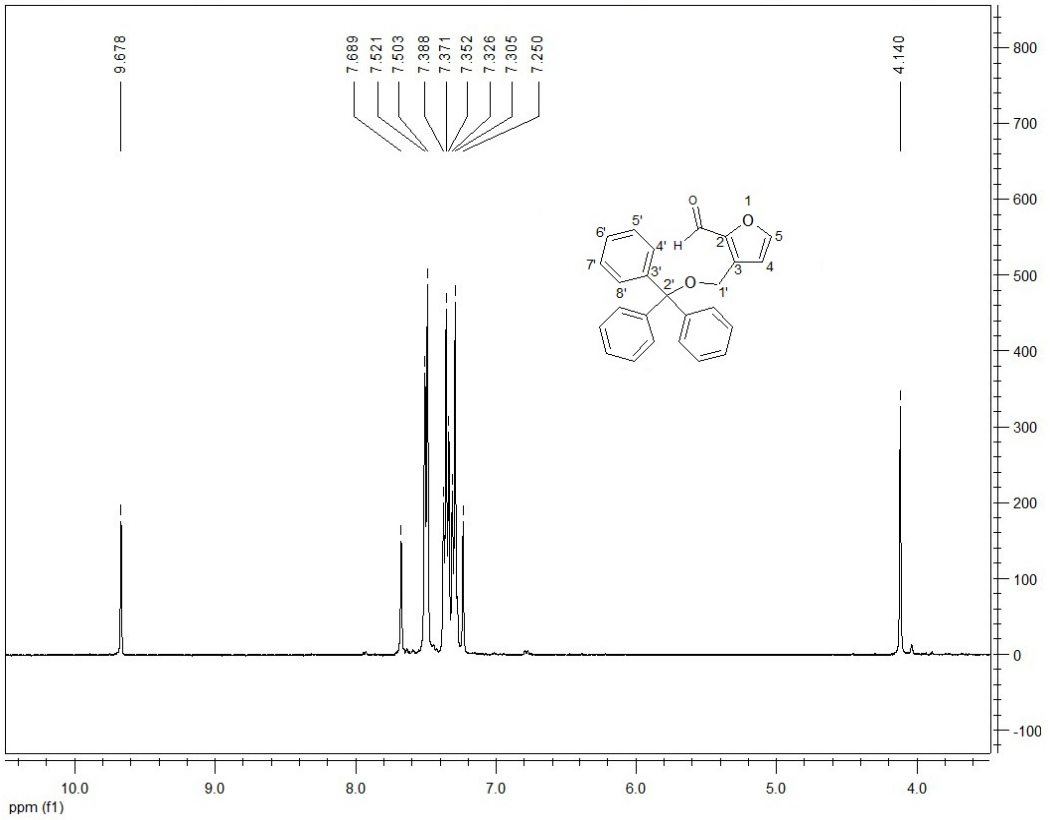
2-(3,3-Dimethylbutanoyl)-4-[(trityloxy)methyl]furan 6c

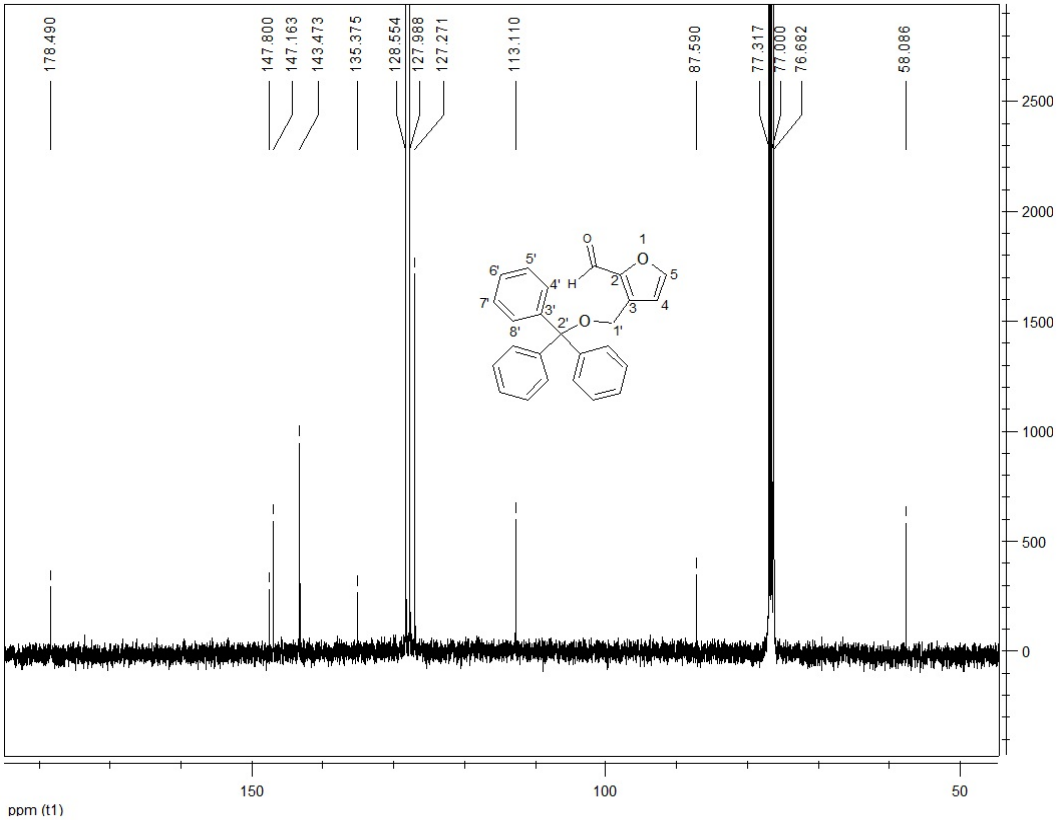




 ©AUTHOR(S)

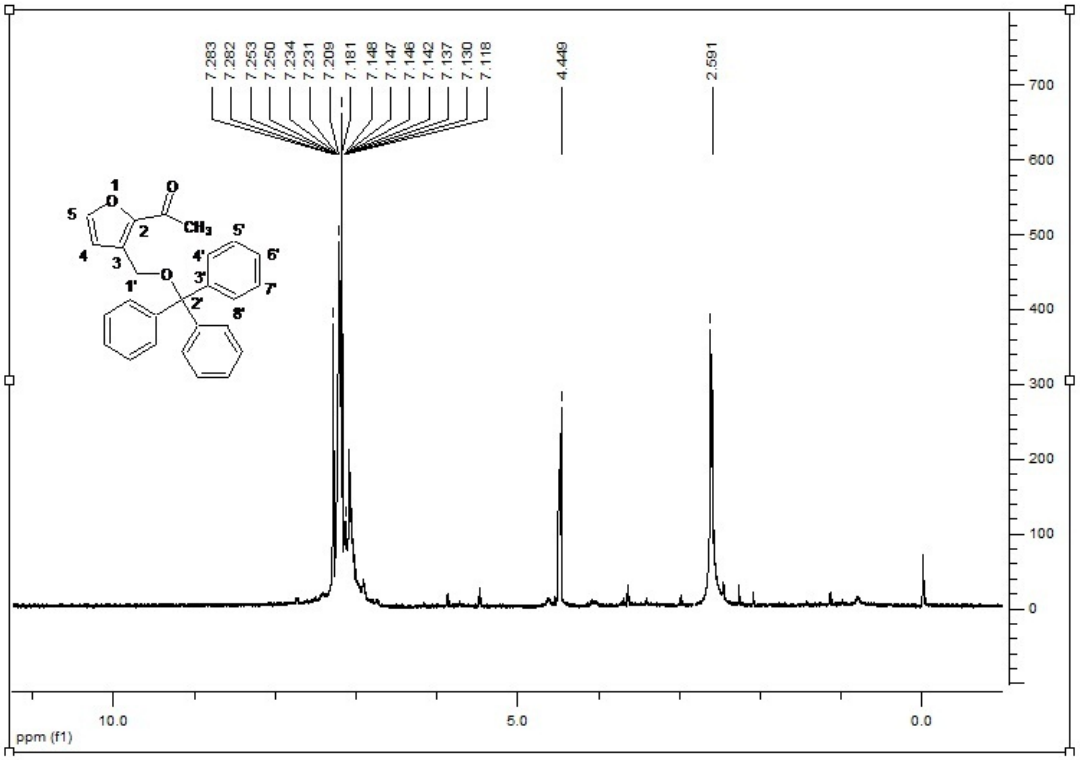
3-[(Trityloxy)methyl]furan-2-carbaldehyde 7a

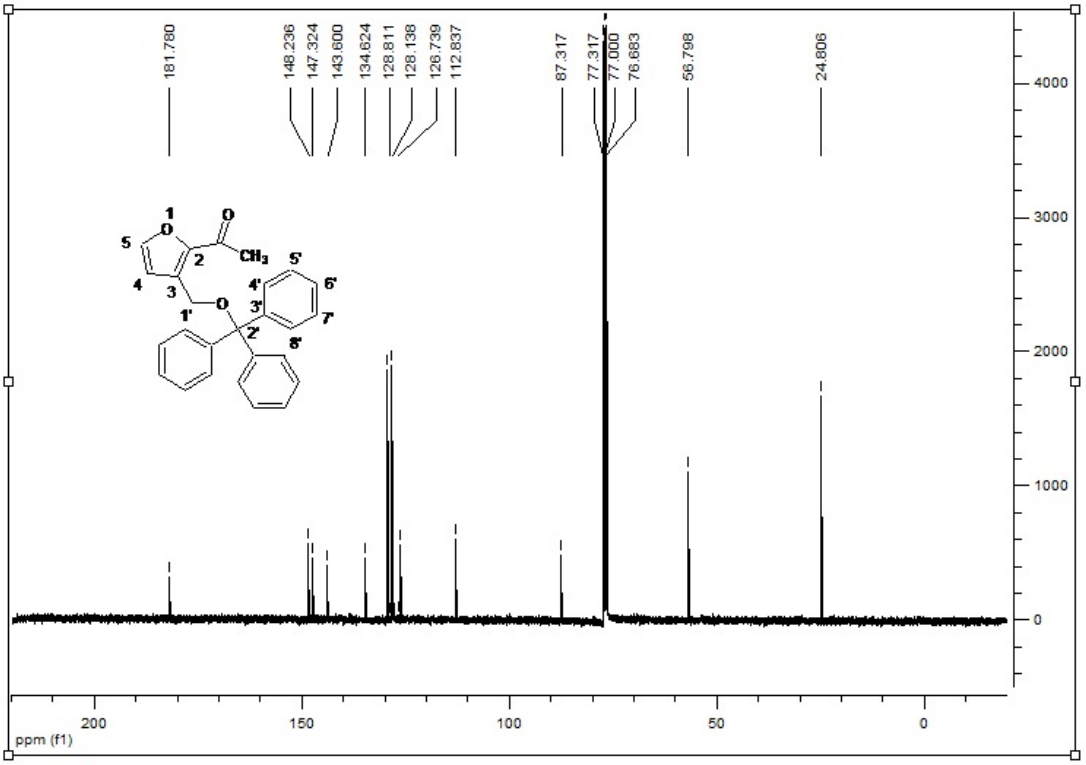




 ©AUTHOR(S)

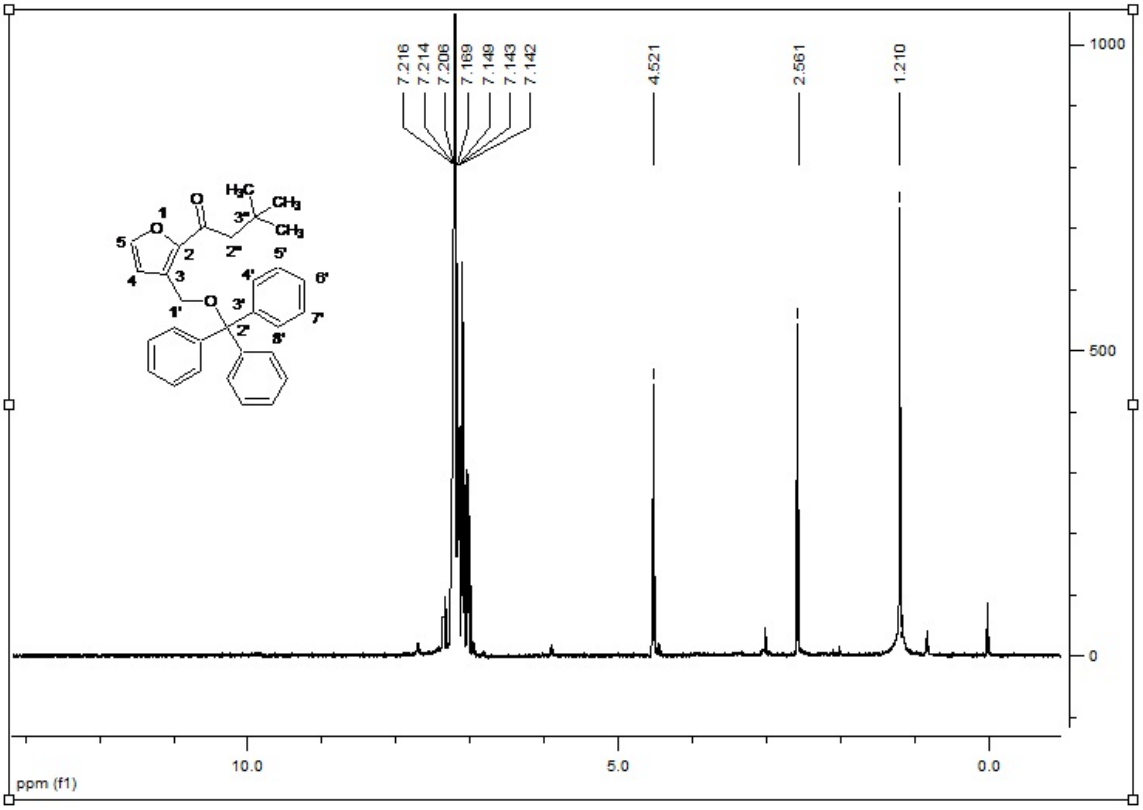
2-acetyl-3-[(trityloxy)methyl]furan 7b

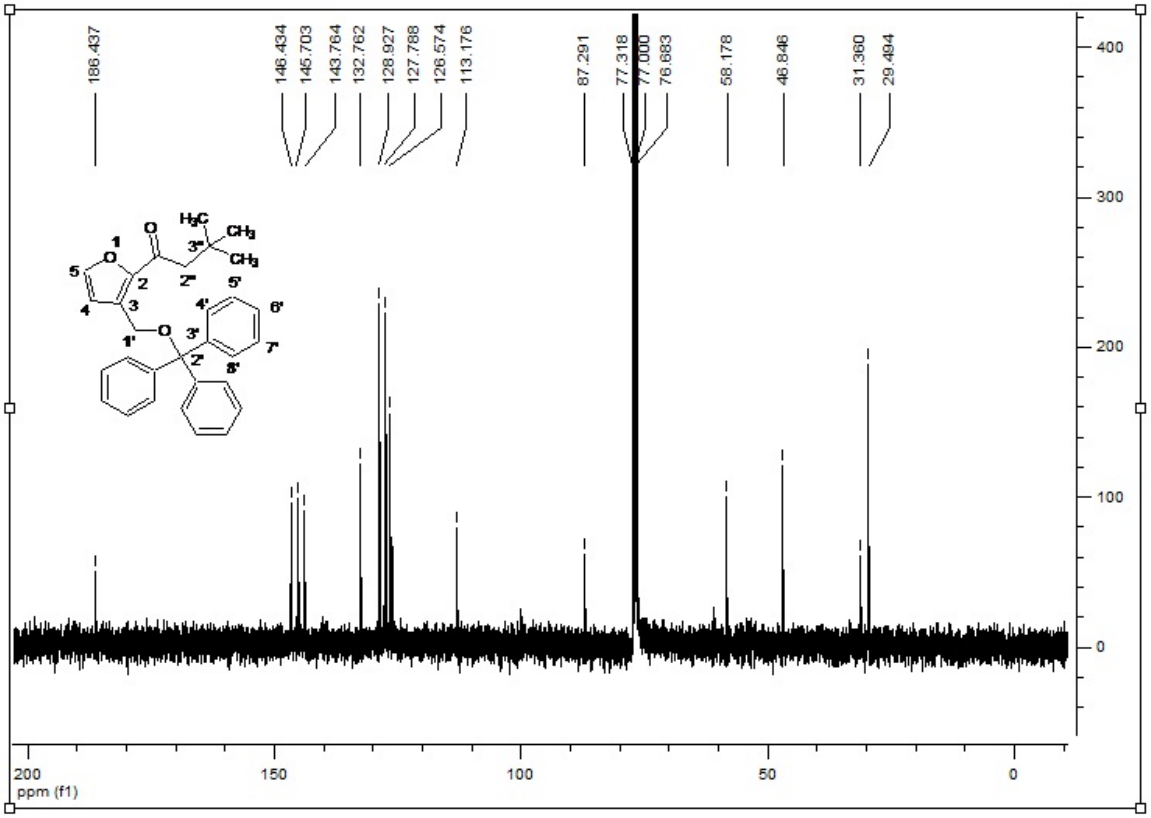




 ©AUTHOR(S)

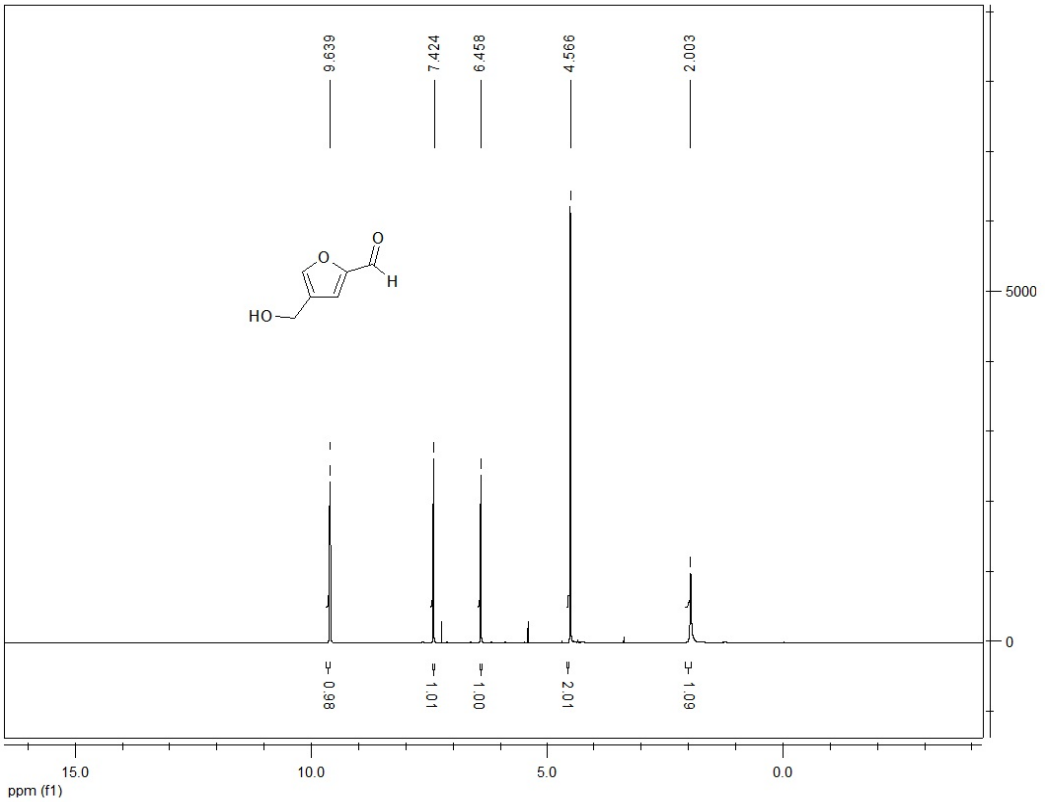
2-(3,3-dimethylbutanoyl)-3-[(trityloxy)methyl]furan 7c

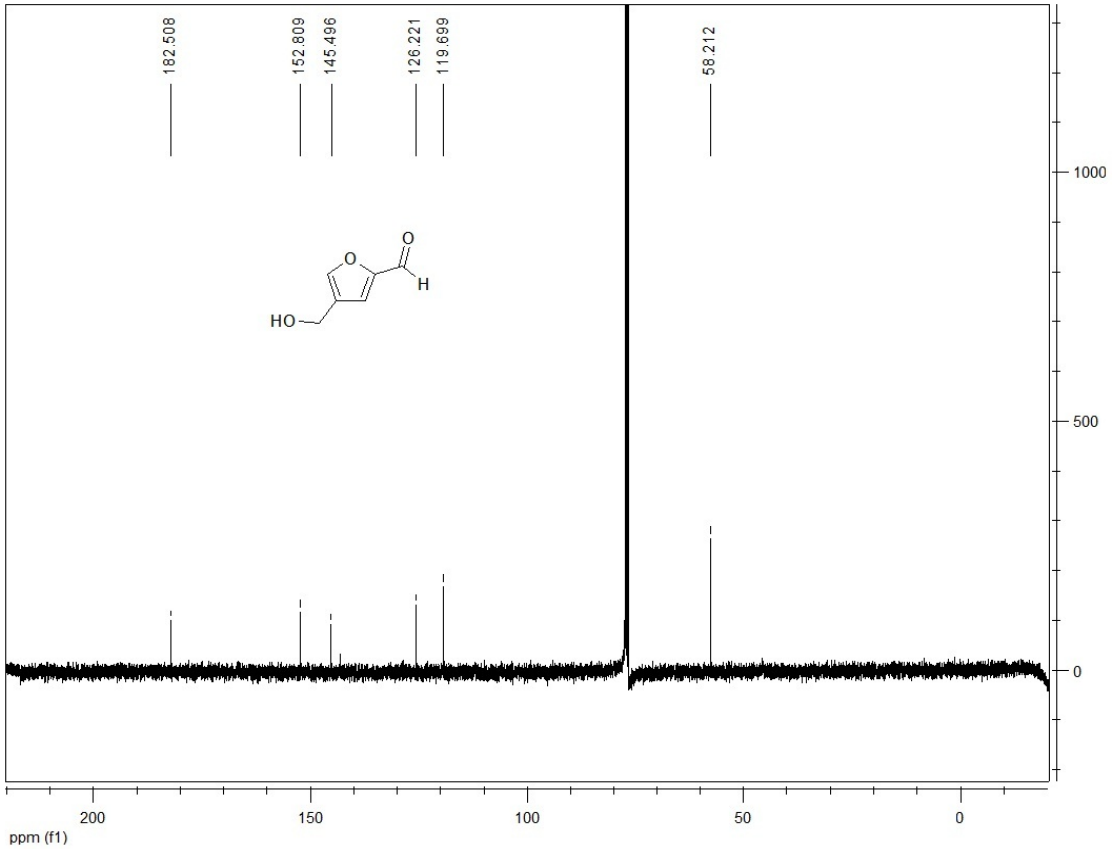




 ©AUTHOR(S)

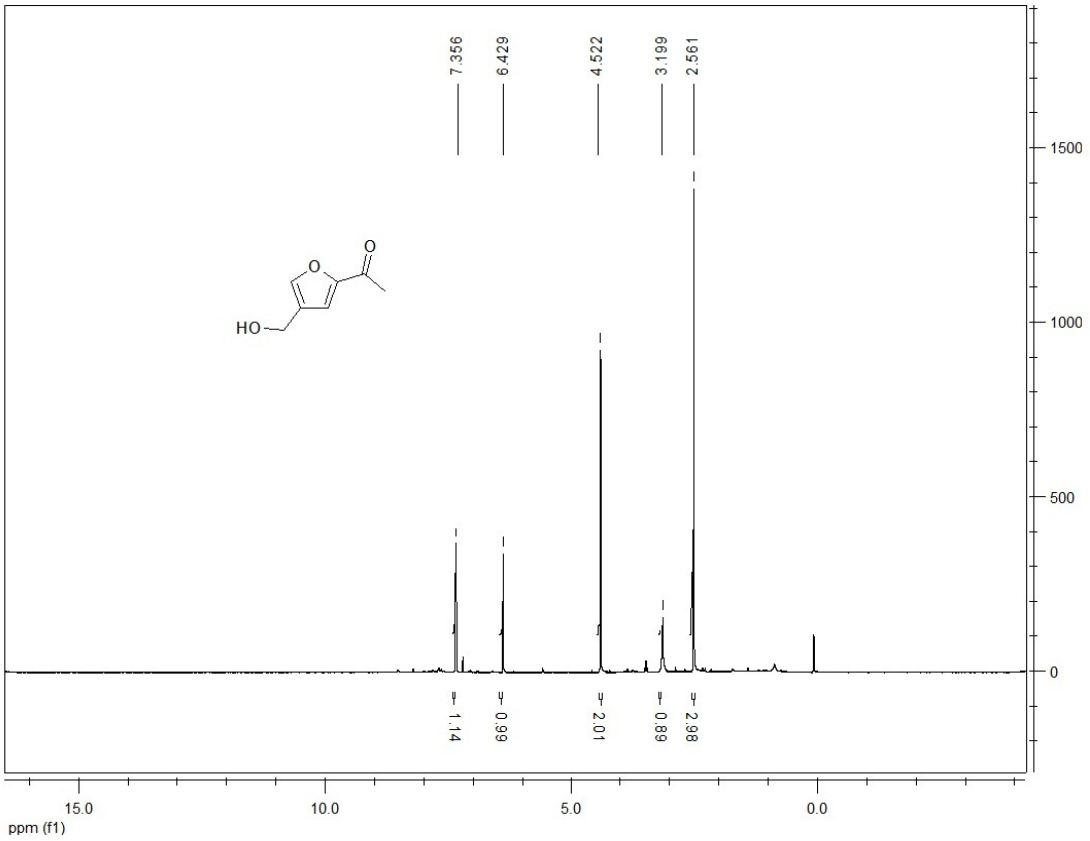
4-(Hydroxymethyl)furan-2-carbaldehyde 8a

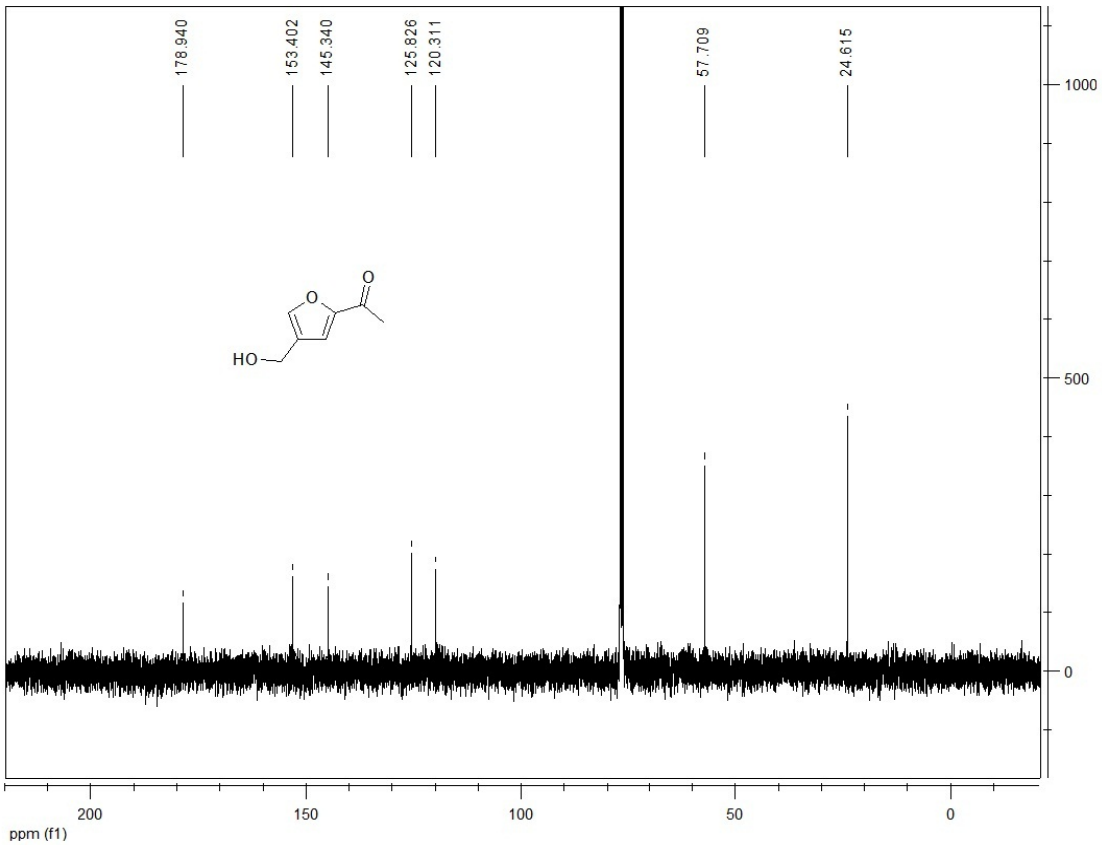




 ©AUTHOR(S)

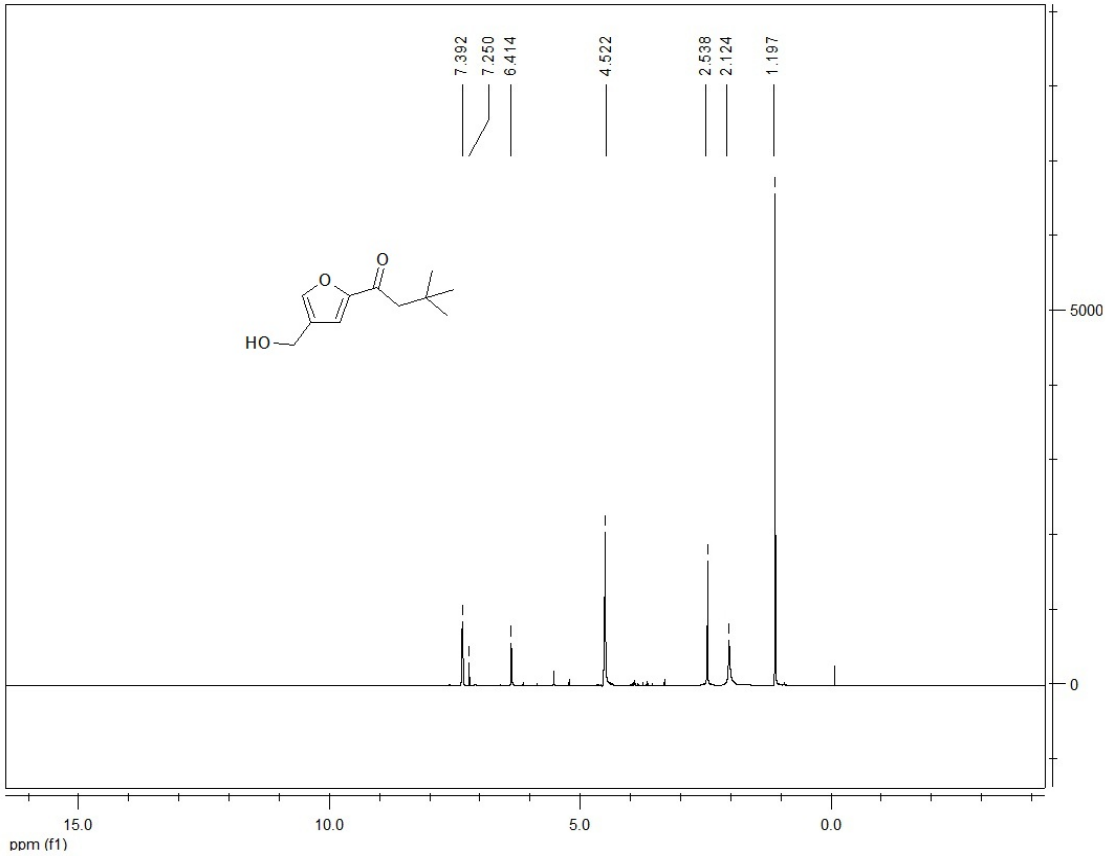
2-Acetyl-1-[4-(Hydroxymethyl)furan 8b

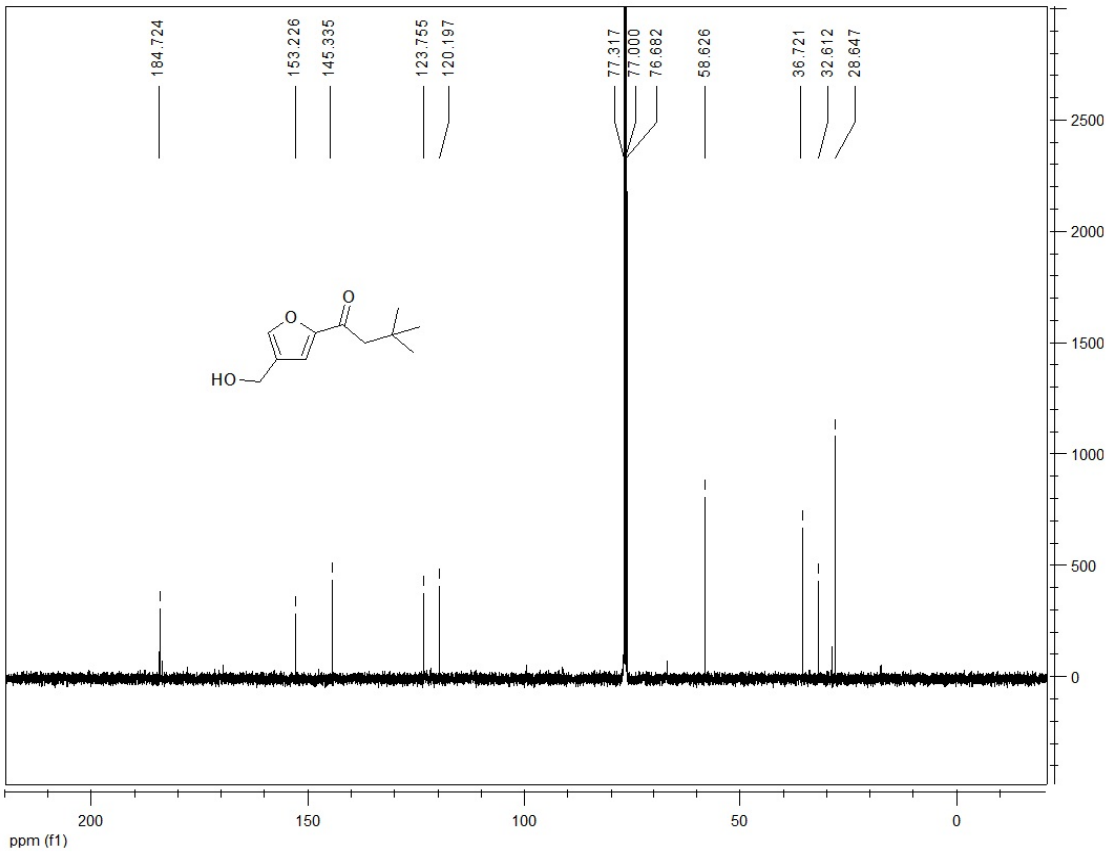




 ©AUTHOR(S)

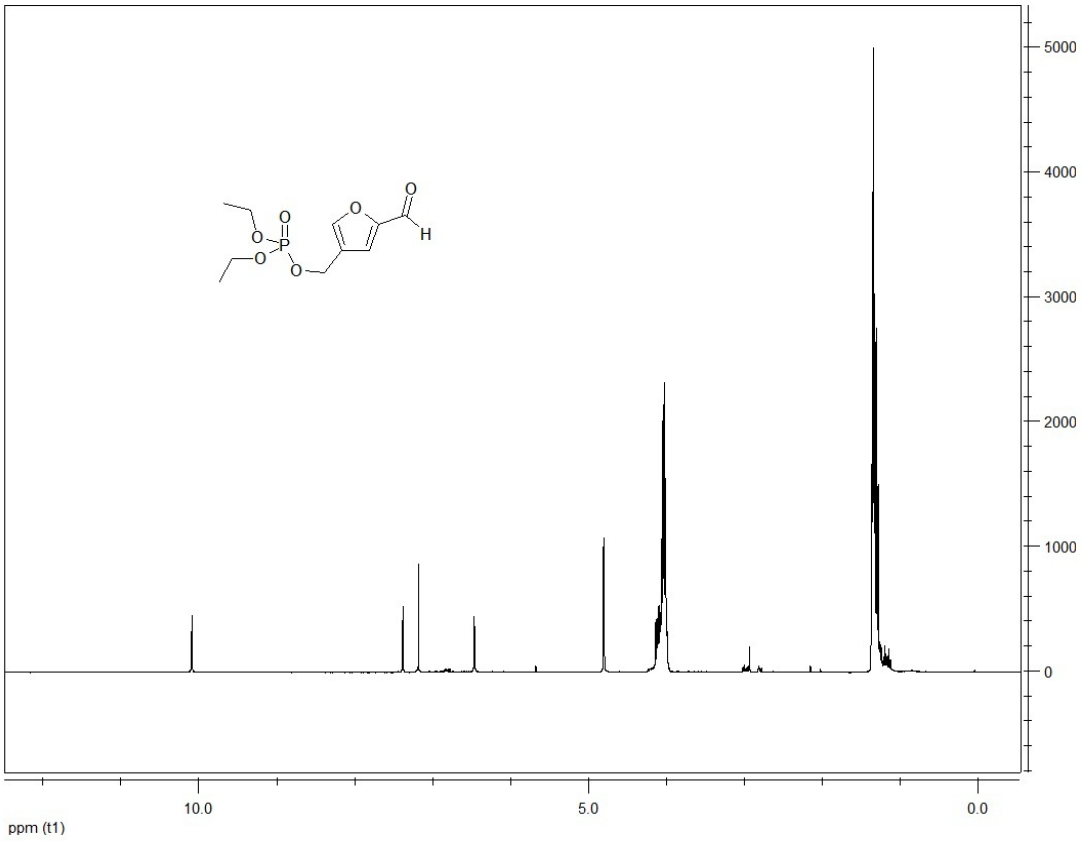
2-(3,3-Dimethylbutanoyl)-1-[4-(hydroxymethyl)]furan 8c

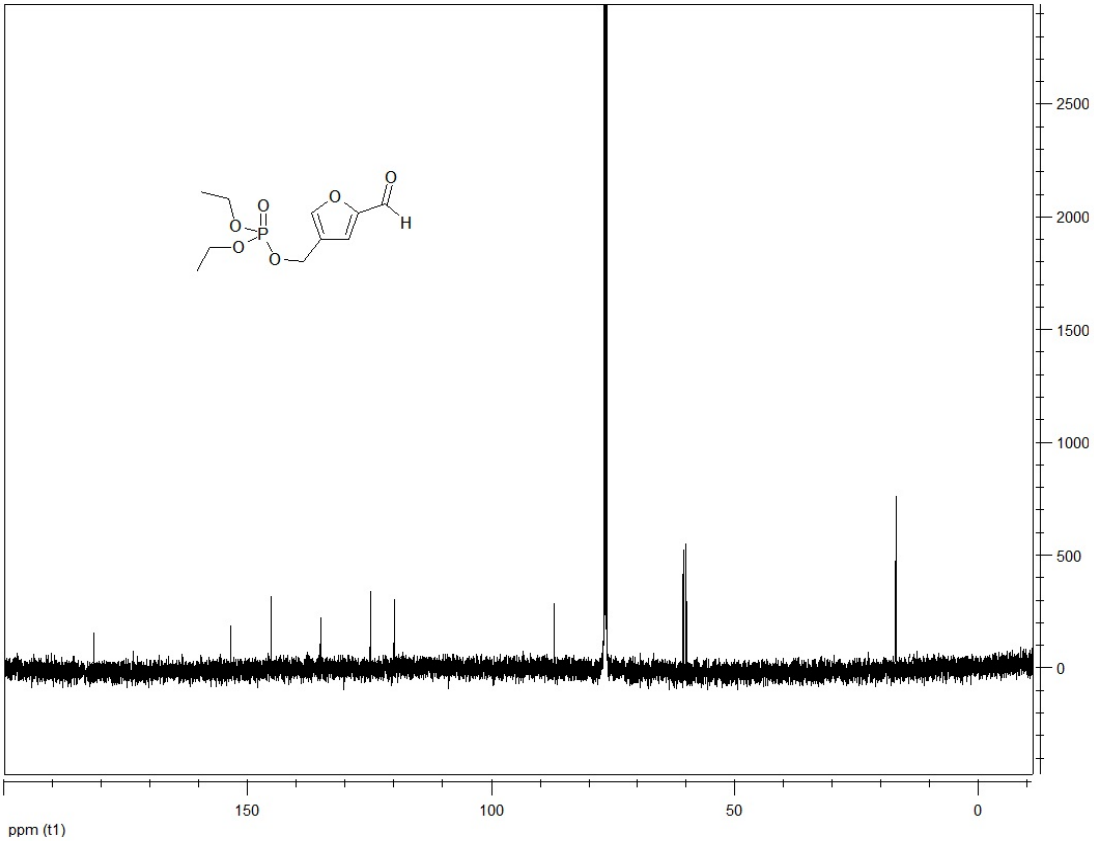




 ©AUTHOR(S)

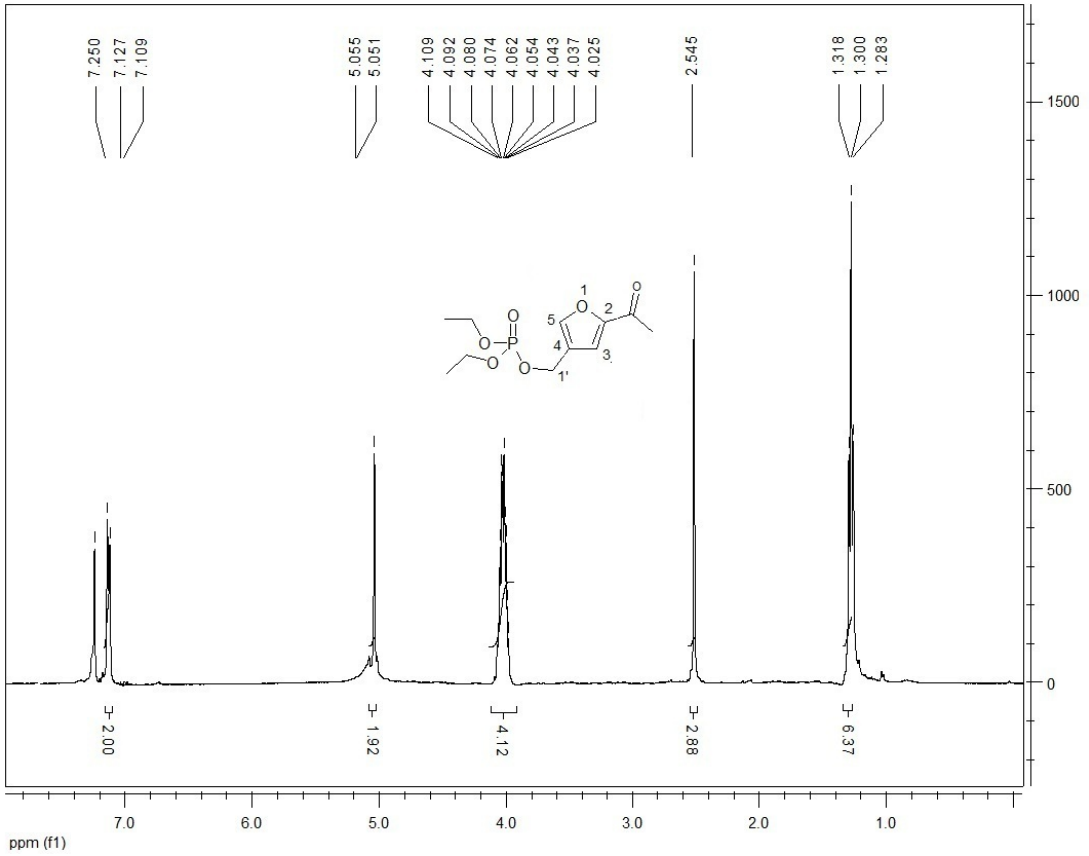
Diethyl (2-formylfuran-4-yl)methyl phosphate 9a

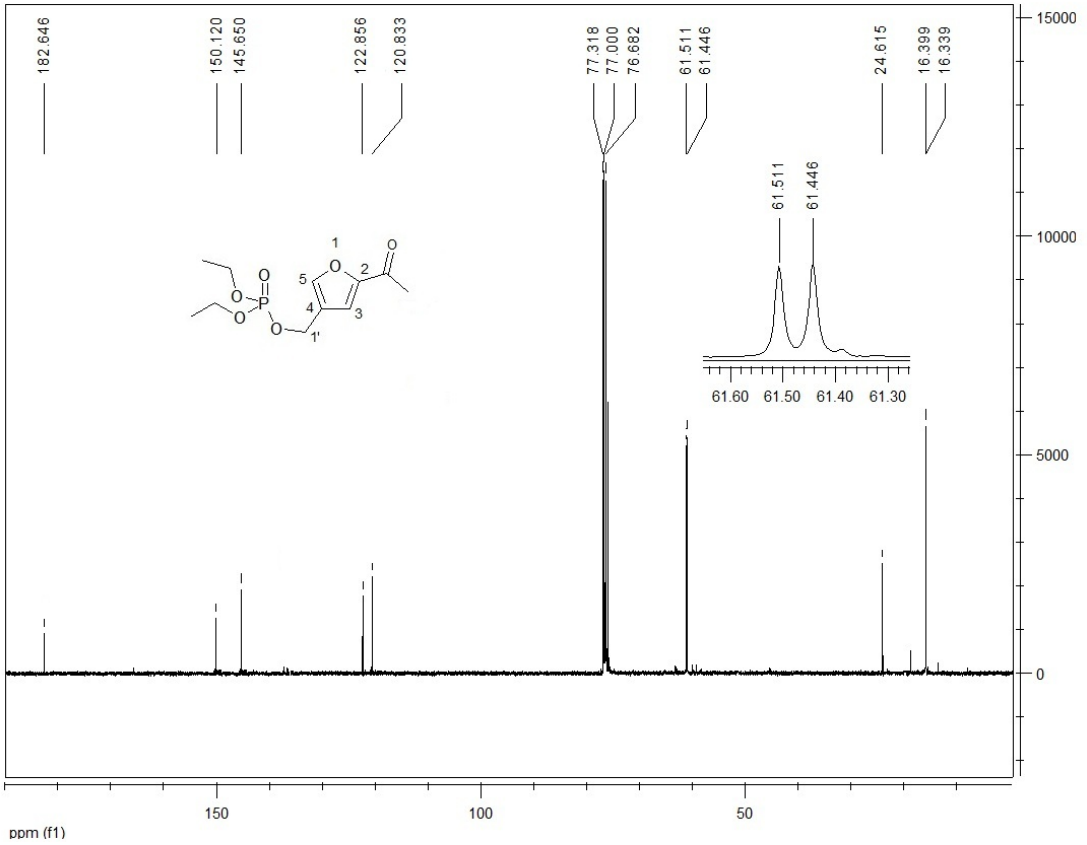




©AUTHOR(S)

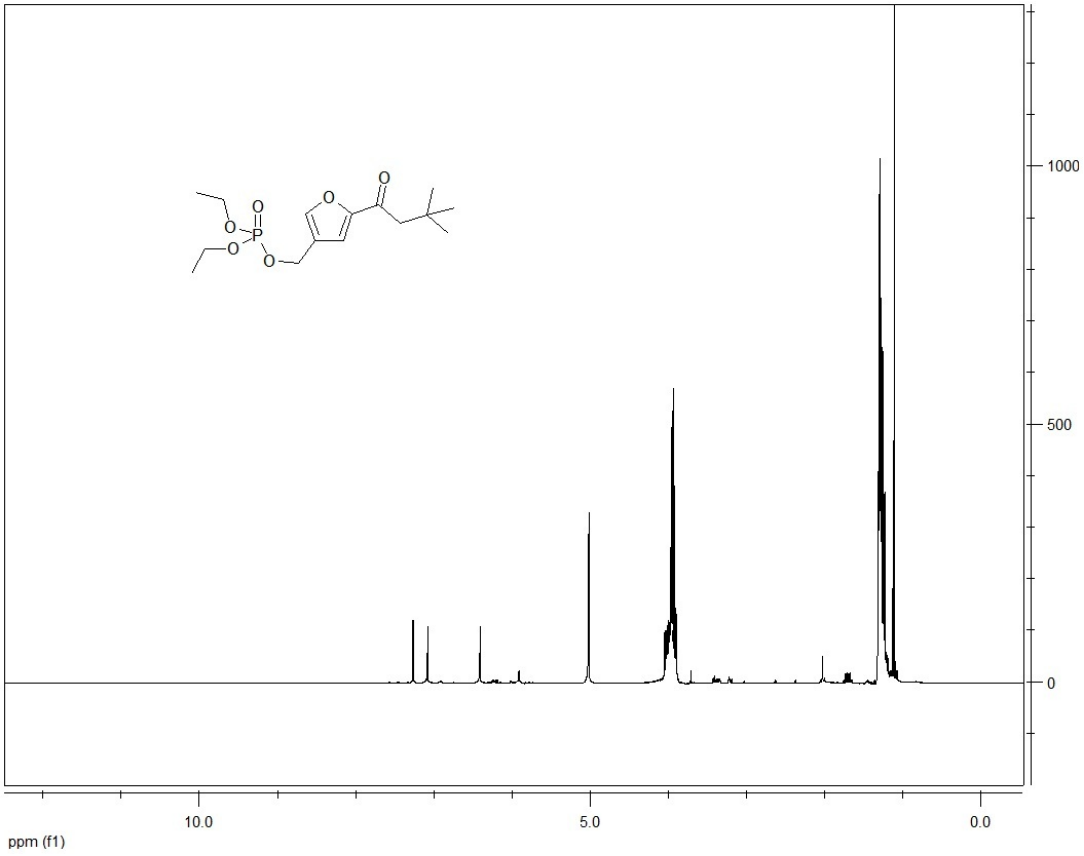
(2-Acetylfuran-4-yl)methyl diethyl phosphate 9b

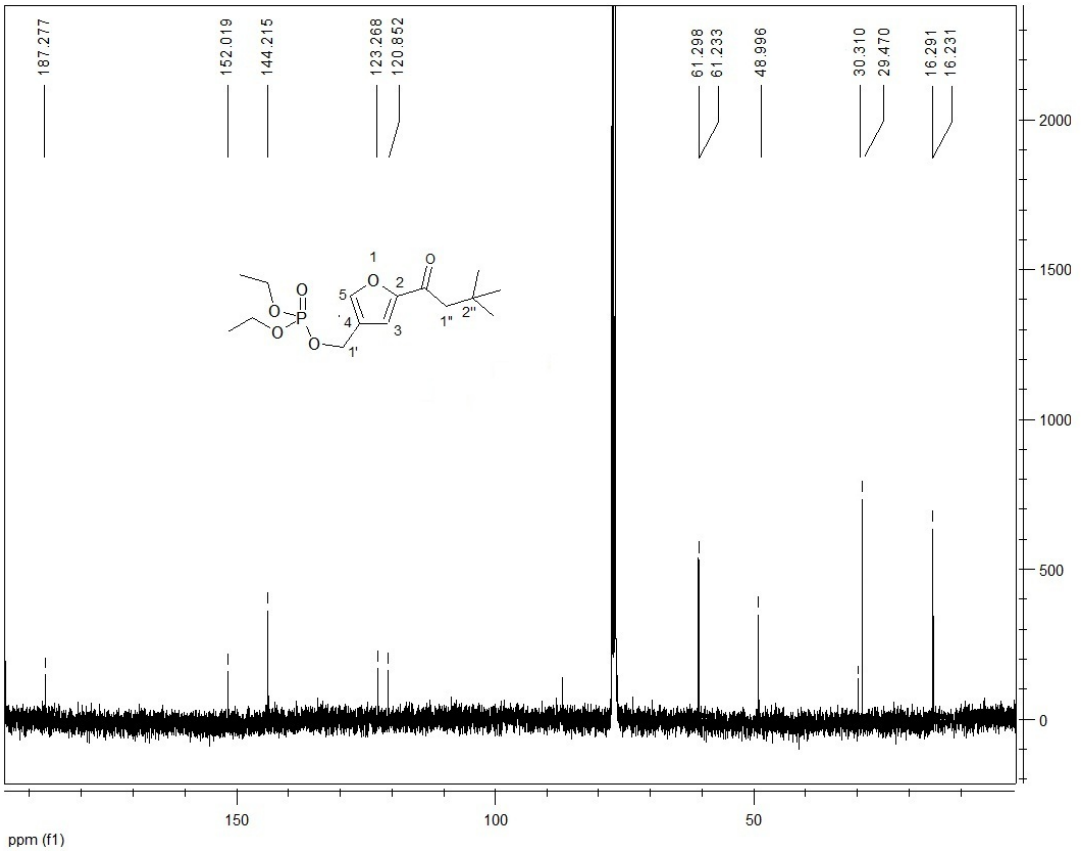




 ©AUTHOR(S)

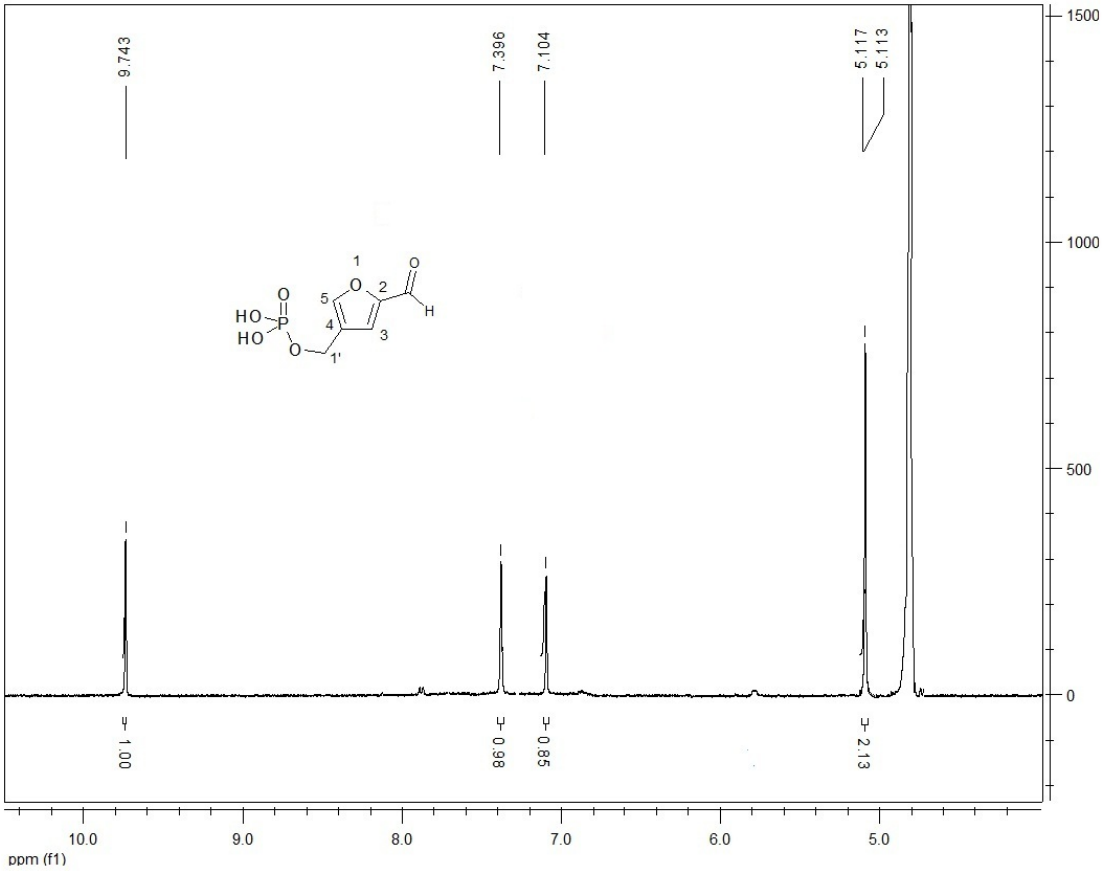
Diethyl [2-(3,3-dimethylbutanoyl)furan-4-yl]methyl phosphate 9c

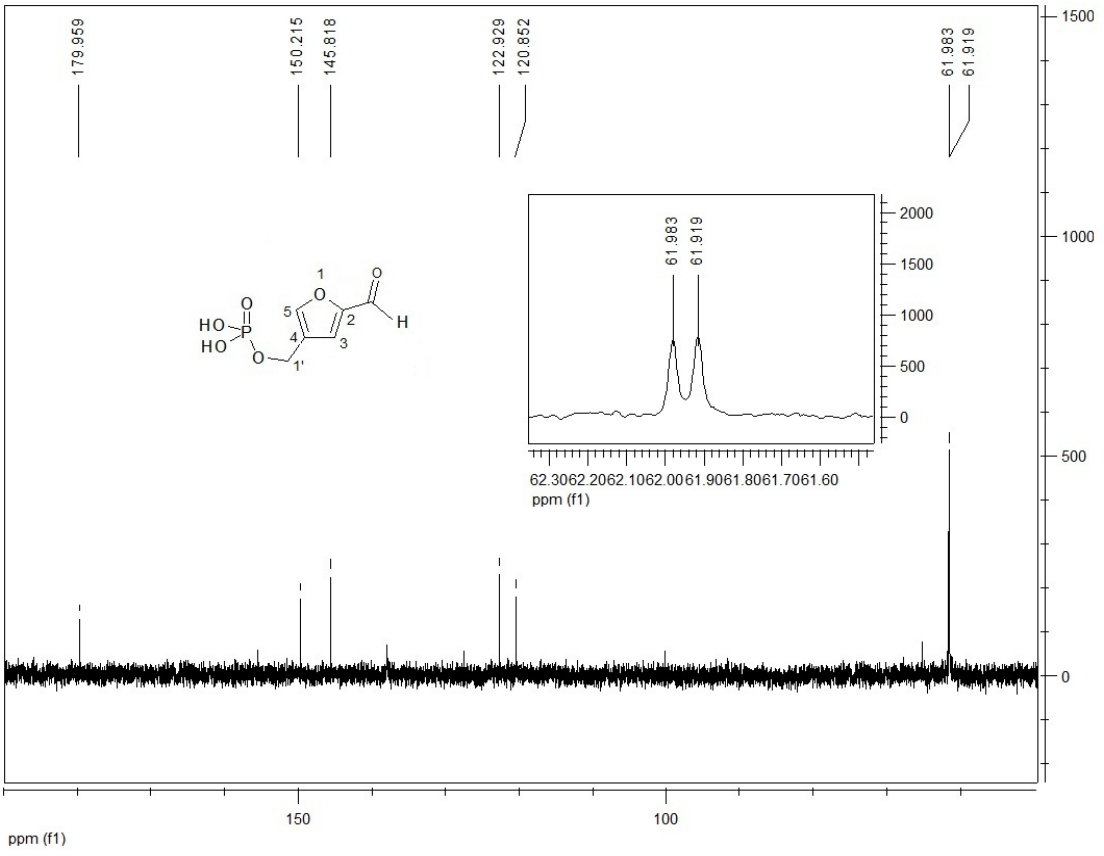




 ©AUTHOR(S)

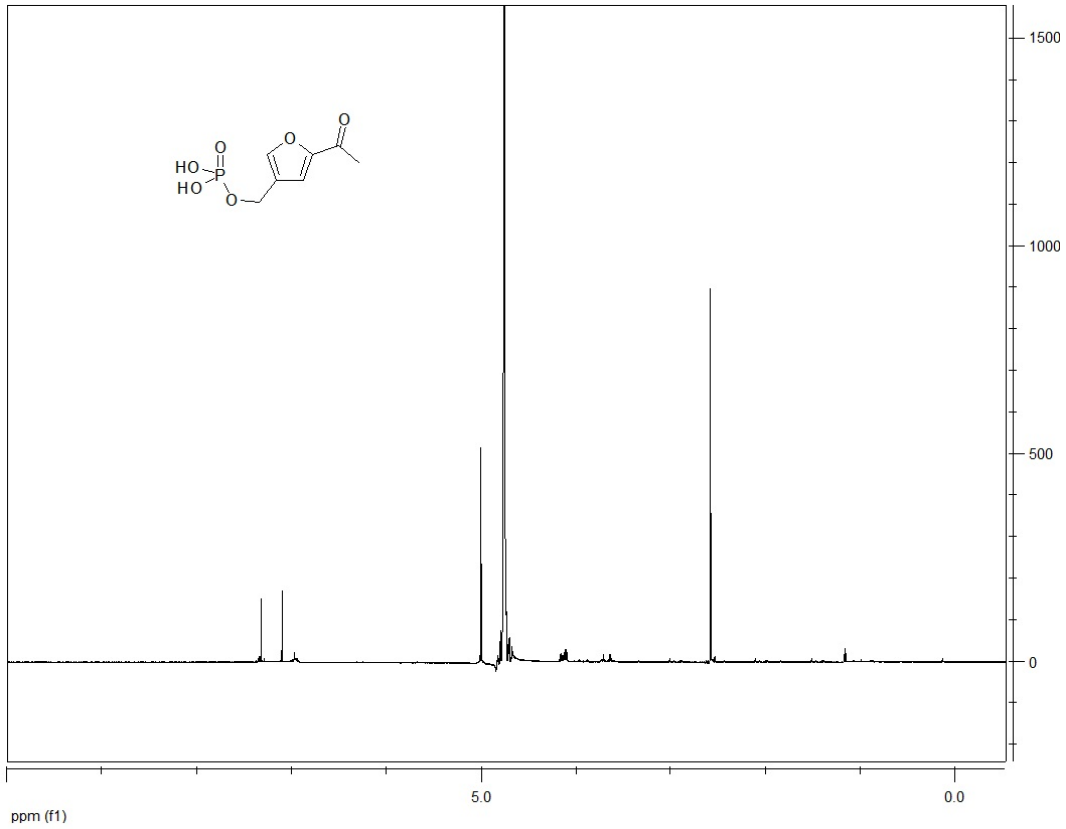
(2-Formylfuran-4-yl)methyl dihydrogen phosphate 10a

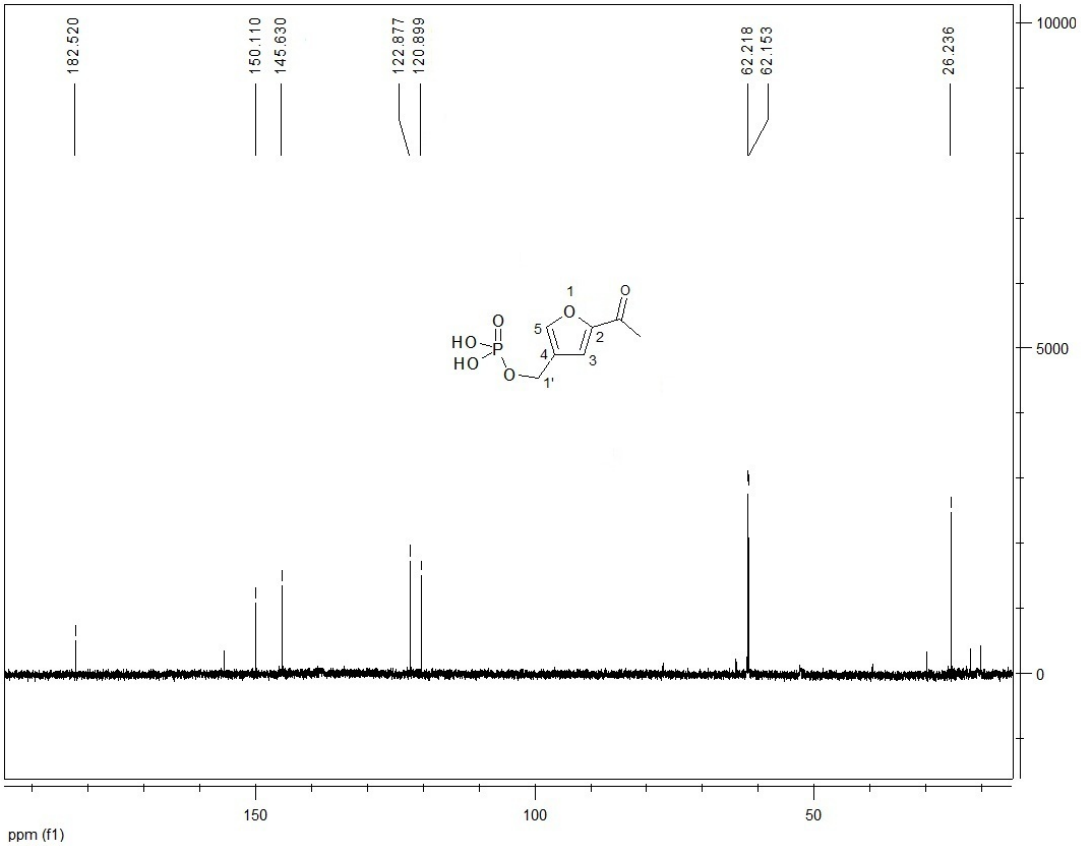




 ©AUTHOR(S)

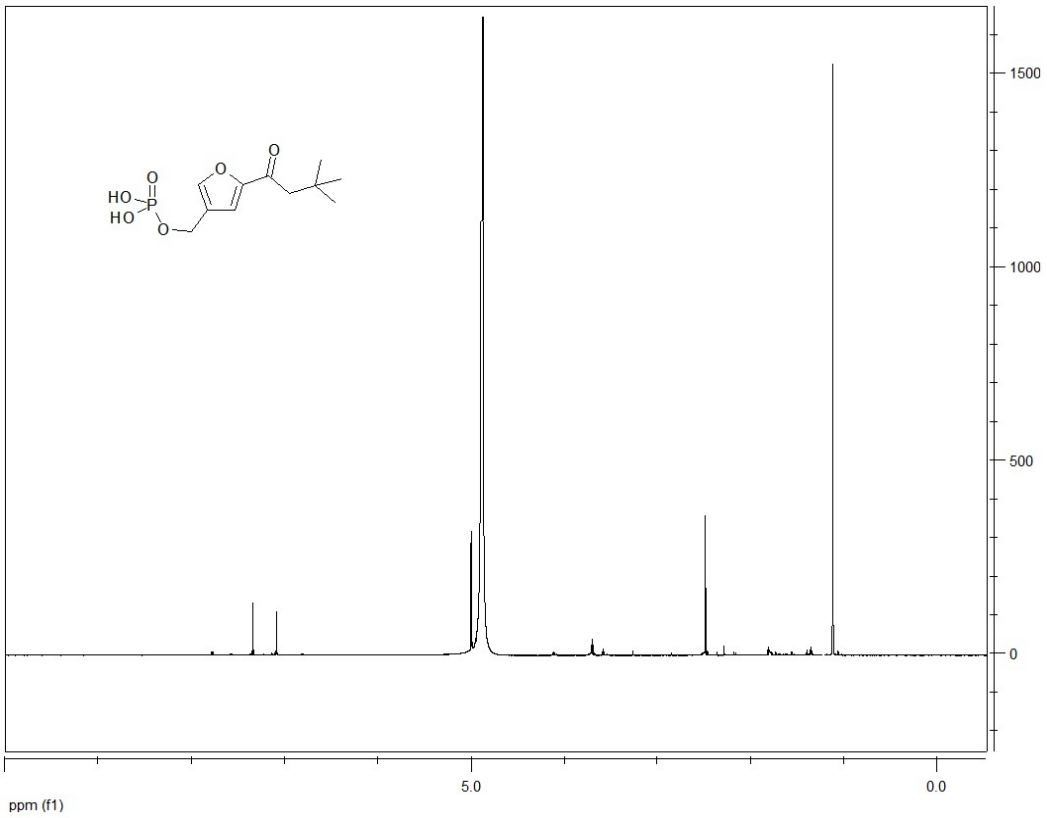
(2-Acetylfuran-4-yl)methyl dihydrogen phosphate 10b

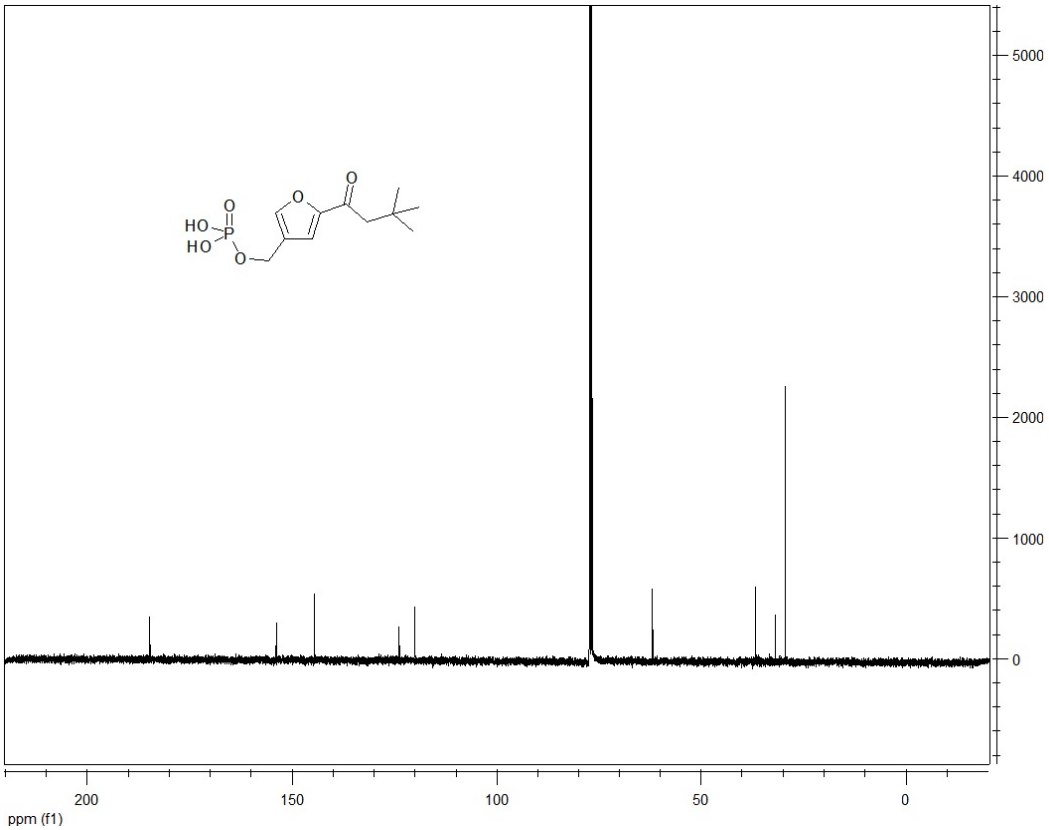




 ©AUTHOR(S)

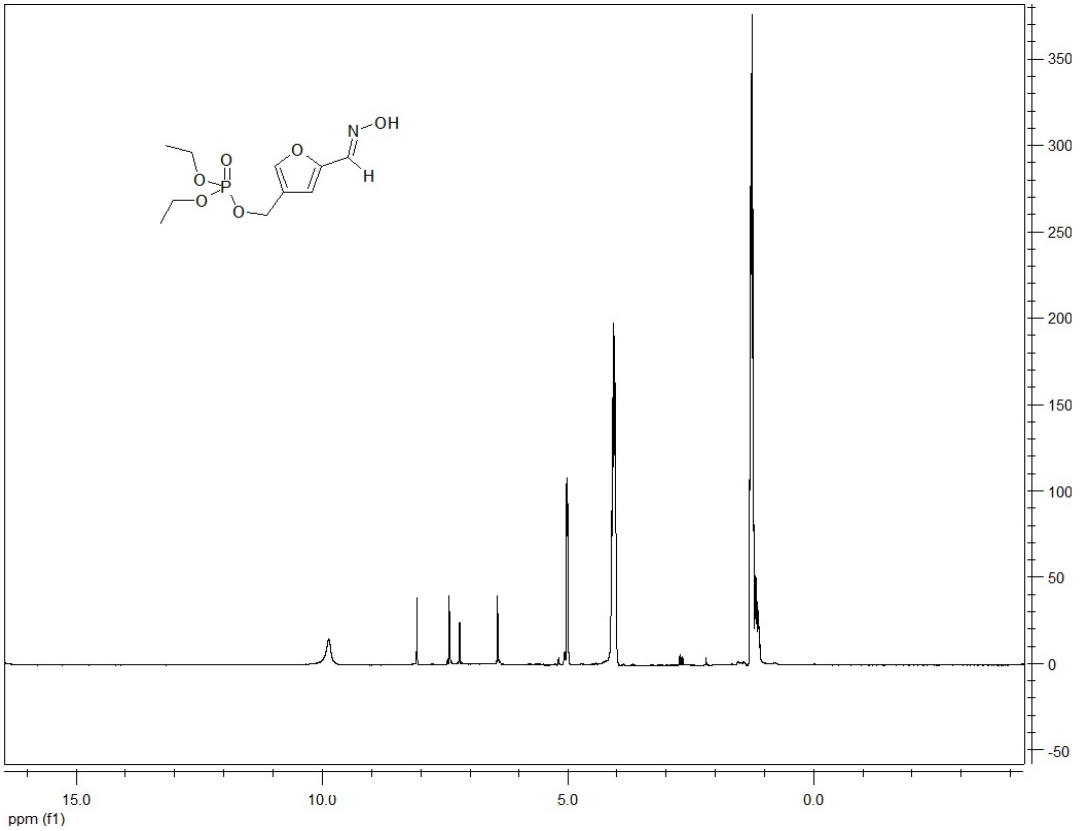
[2-(3,3-Dimethylbutanoyl)furan-4-yl]methyl dihydrogen phosphate 10c

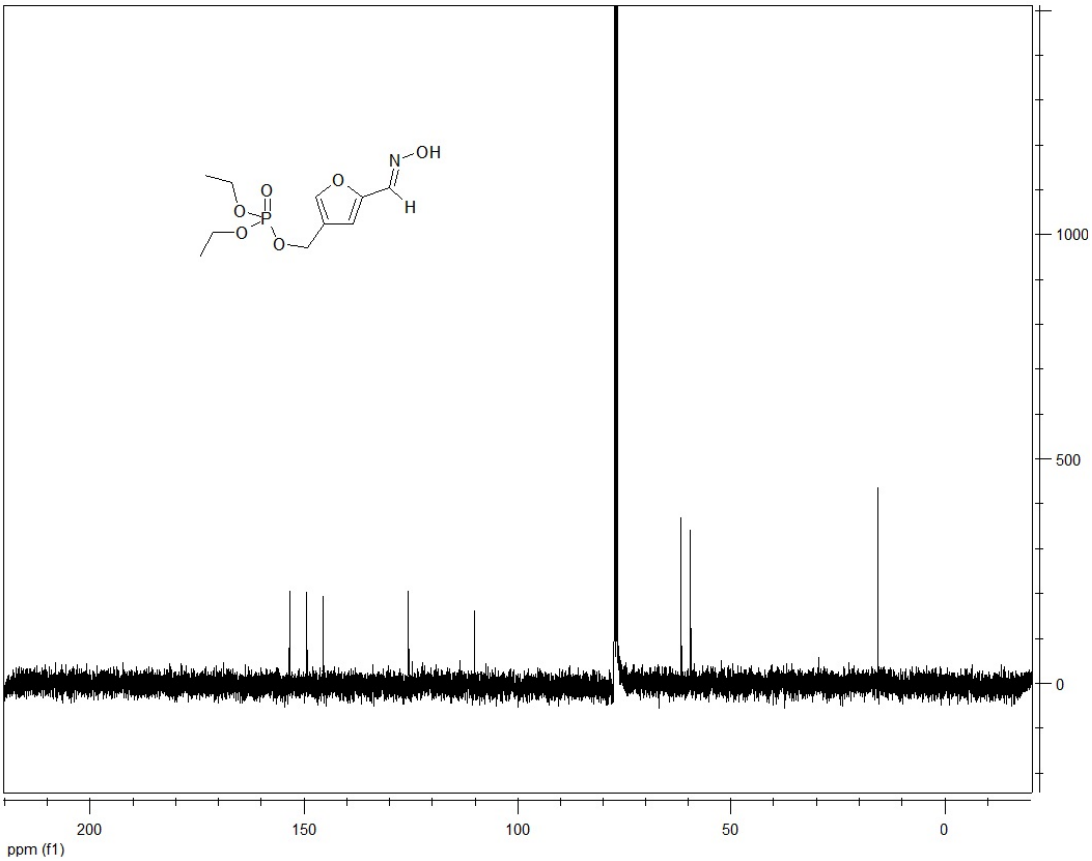




©AUTHOR(S)

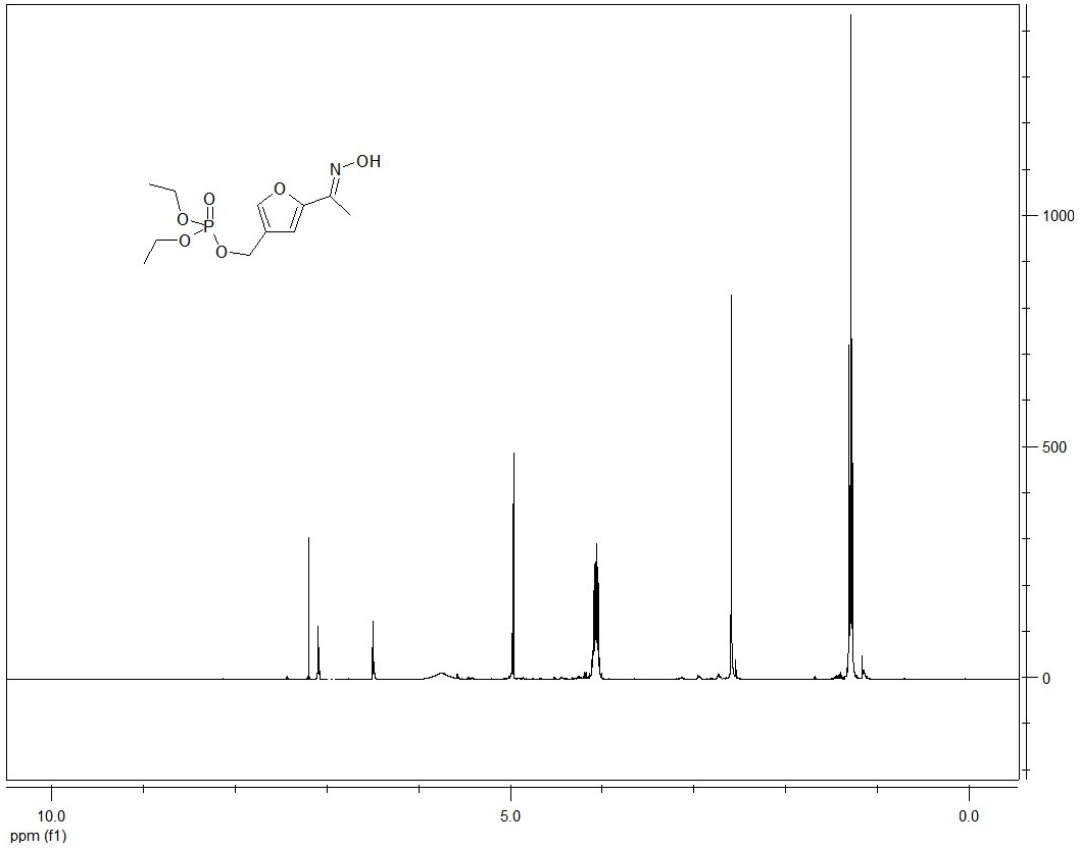
Diethyl {2-[(hydroxyimino)methyl]furan-4-yl}methyl phosphate 11a

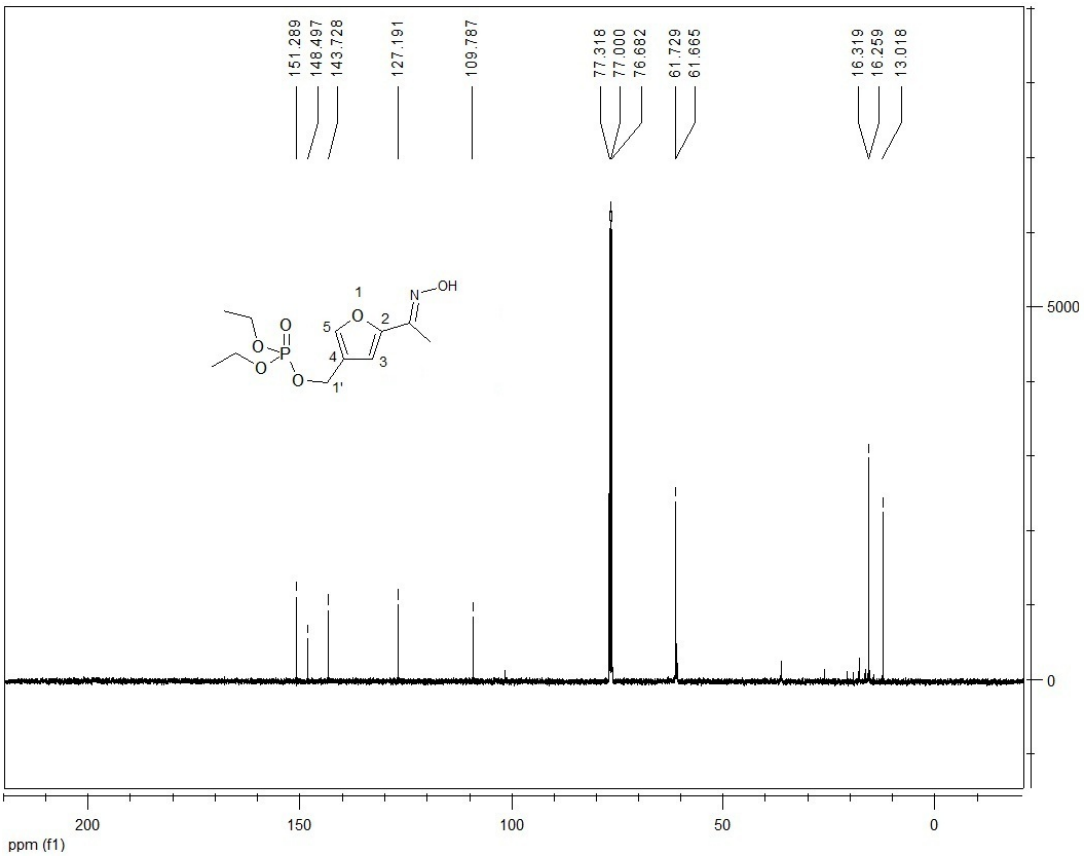




 ©AUTHOR(S)

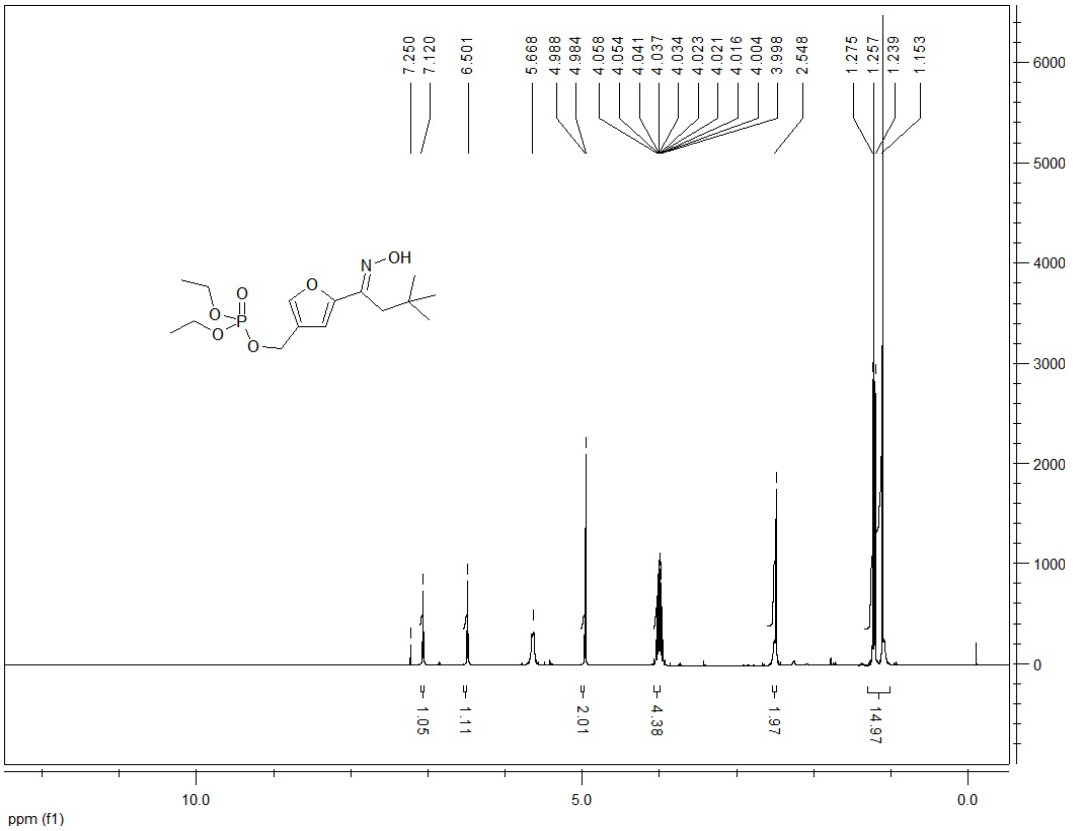
Diethyl {2-[1-(hydroxyimino)ethyl]furan-4-yl}methyl phosphate 11b

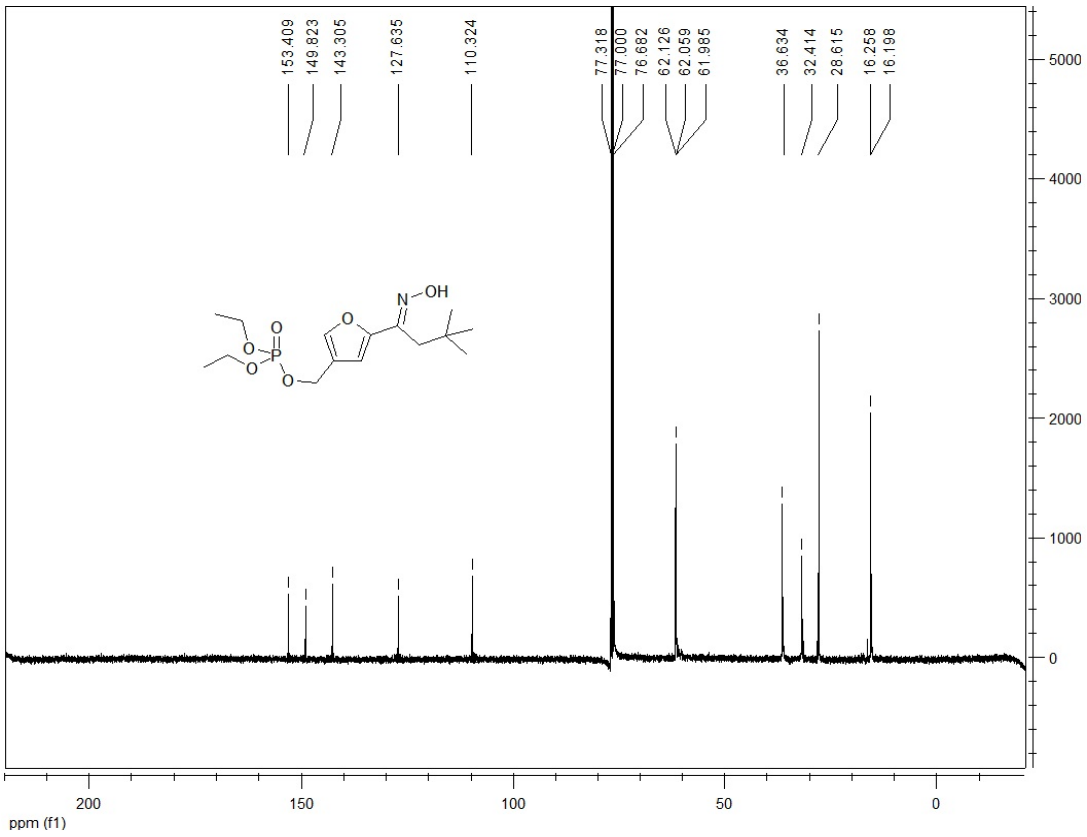




 ©AUTHOR(S)

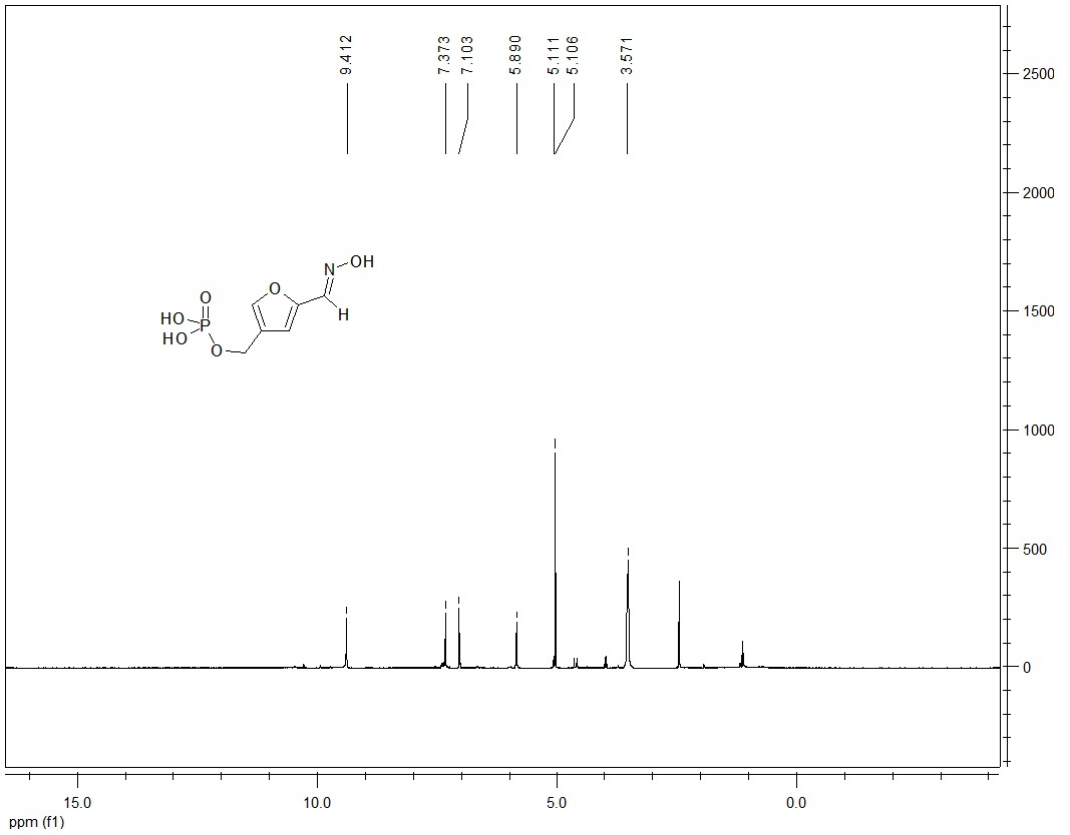
Diethyl {2-[1-(hydroxyimino)-3,3-dimethylbutyl]furan-4-yl}methyl phosphate 11c

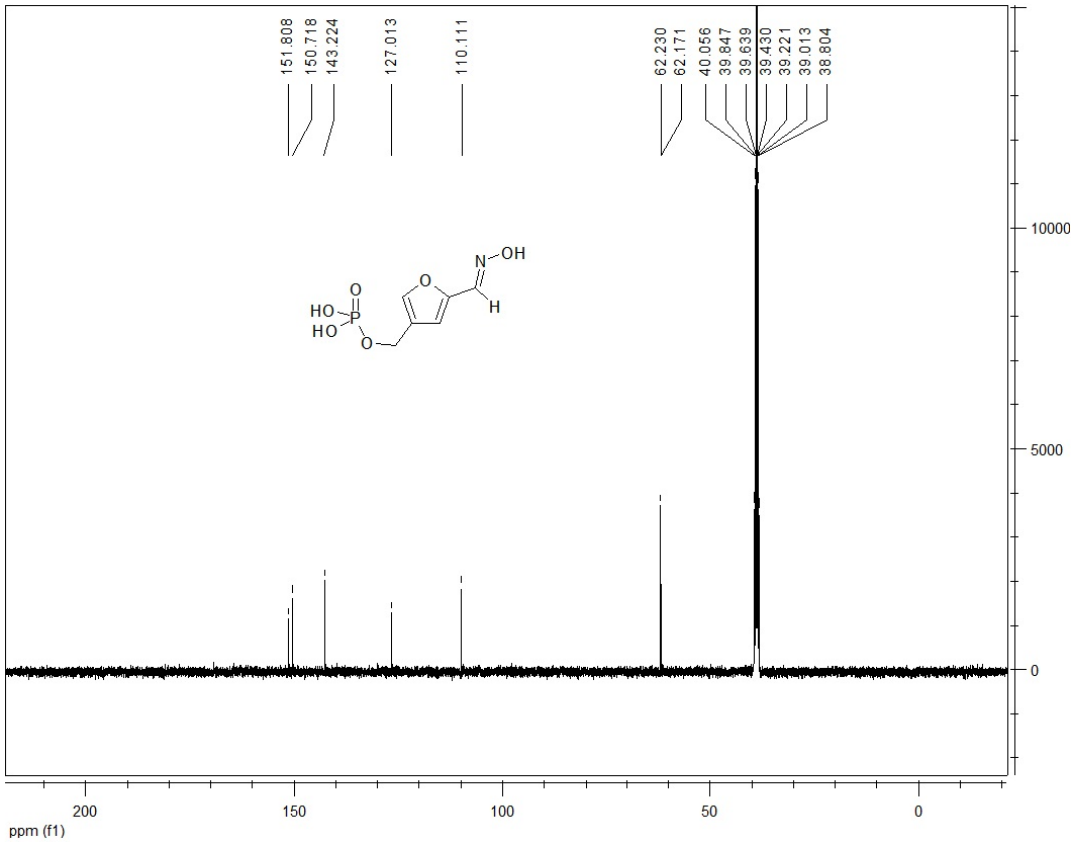




 ©AUTHOR(S)

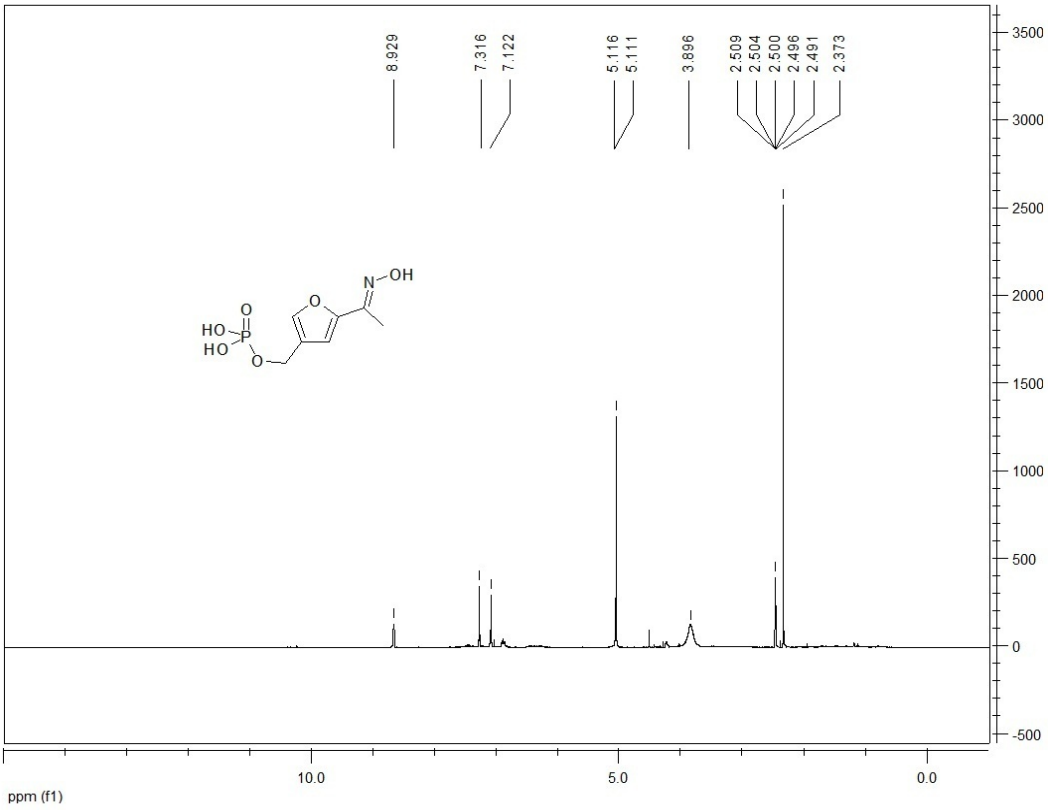
{2-[(Hydroxyimino)methyl]furan-4-yl}methyl dihydrogen phosphate 3ª

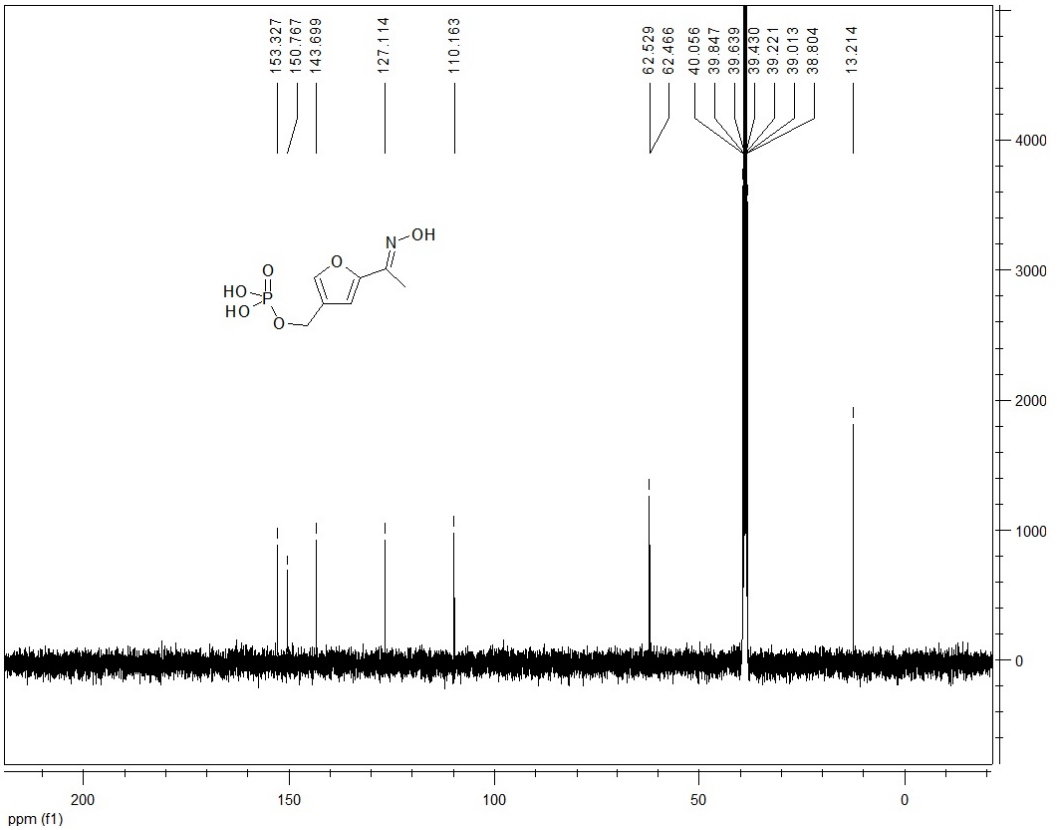




 ©AUTHOR(S)

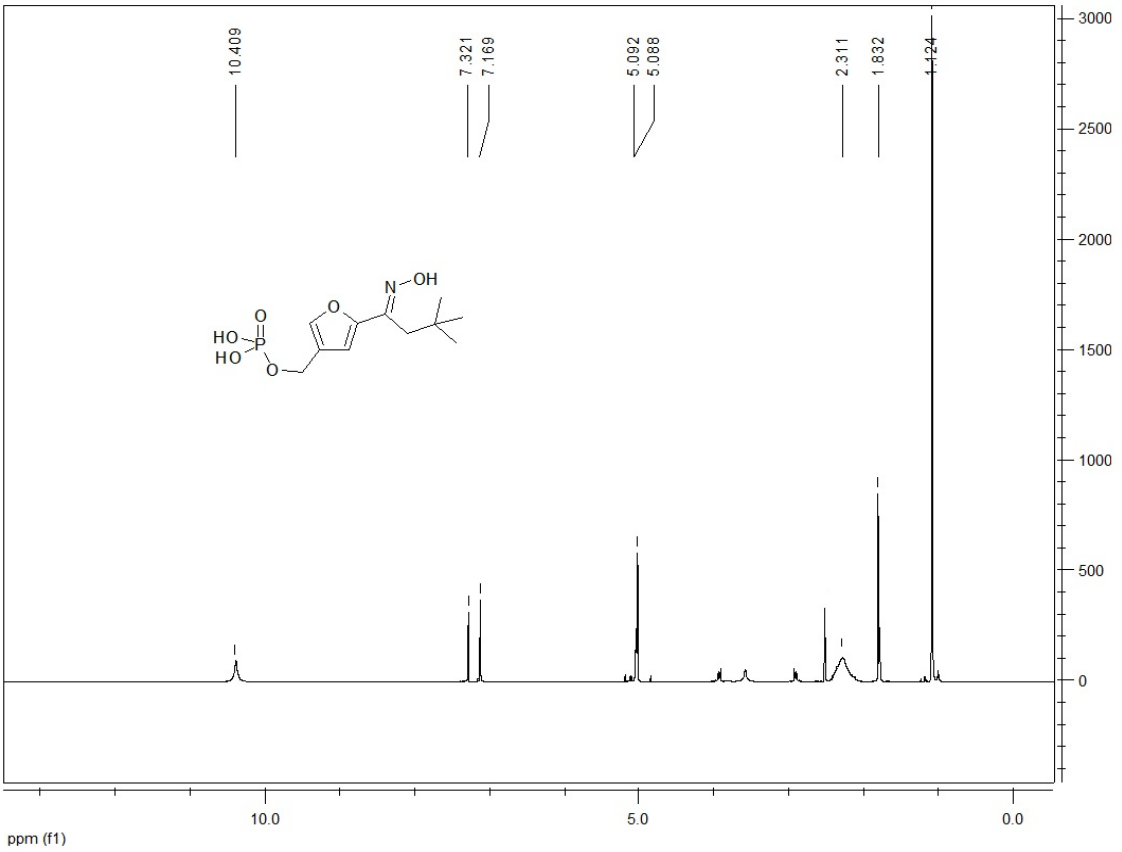
{2-[1-(Hydroxyimino)ethyl]furan-4-yl}methyl dihydrogen phosphate 3b

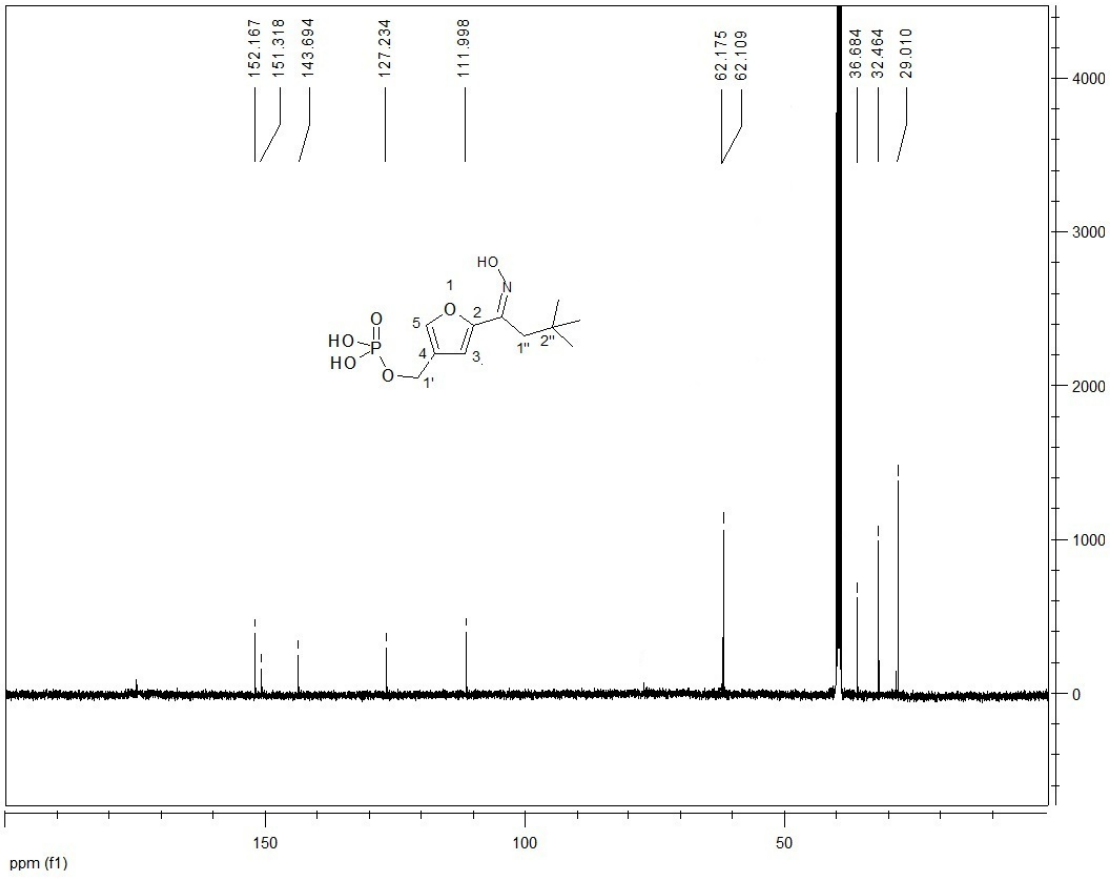




 ©AUTHOR(S)

{2-[1-(Hydroxyimino)-3,3-dimethylbutyl]furan-4-yl}methyl dihydrogen phosphate 3c





 ©AUTHOR(S)

II. *In Silico* Modelling Data

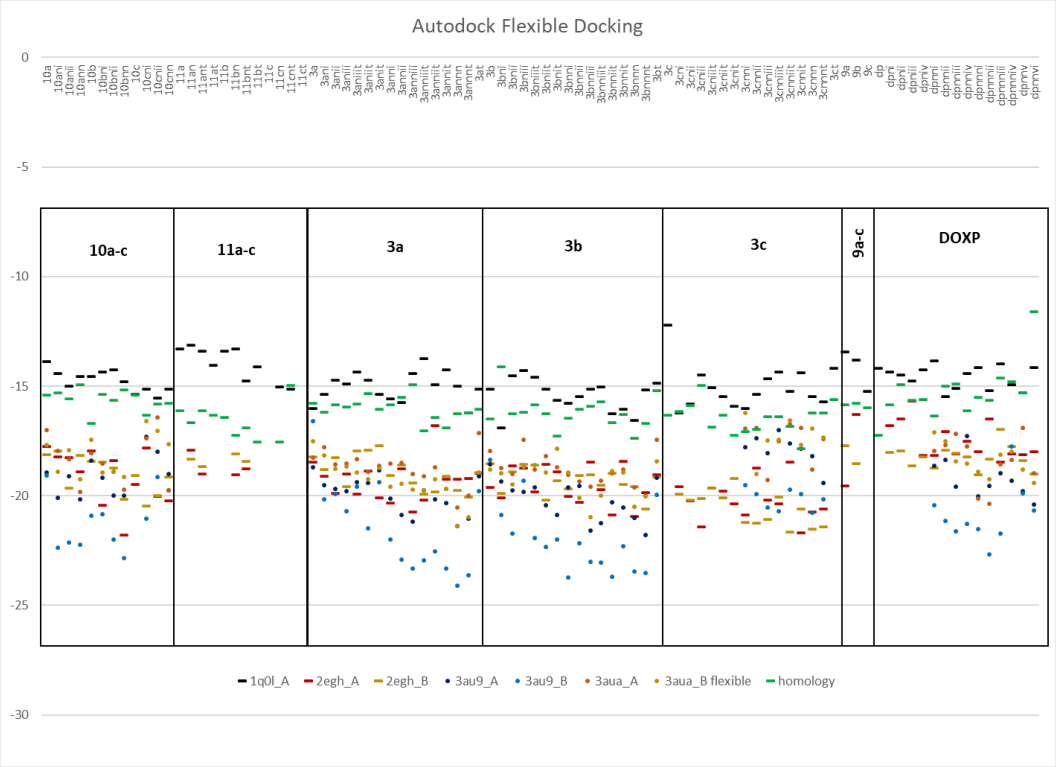


Figure 1. Binding energies from Autodock flexible docking. Dashes indicate binding to EcDXR structures; circles to PfDXR structures. Binding is relatively poor to 1QOL across all compounds. In general **3c** binds better to the *Ec*DXR structures 2EGH, while **3a** and **3b** bind better to the *Pf*DXR structures 3AUA and 3AU9 in this set of docking experiments.

©AUTHOR(S)

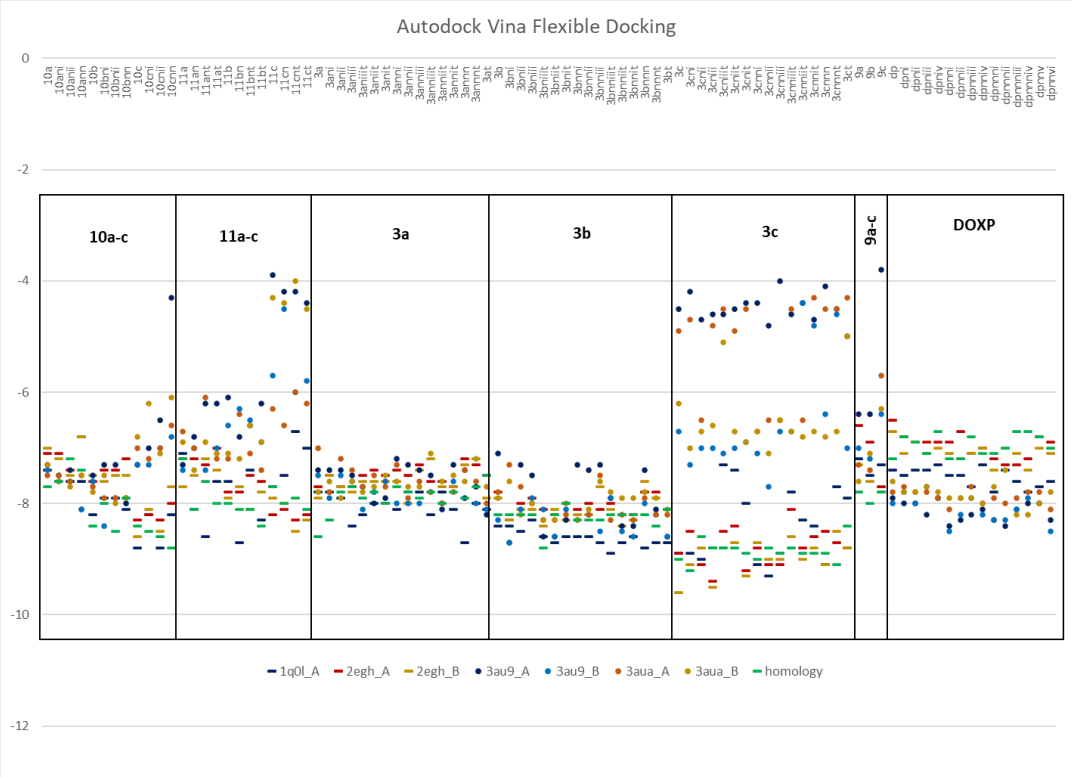


Figure 2. Binding energies for Autodock Vina flexible docking. Circles represent binding energies to *Pf*DXR structures, while dashes indicate binding to *Ec*DXR structures. The potential pro-drugs **11a-c** show variable but high binding energy to most systems; **3a** and **3b** bind well to all structures, while **3c** binds preferentially to *Ec*DXR systems (with the exception of the *Pf*DXR homology model which was modelled on an *Ec*DXR template).

©AUTHOR(S)

Table 1. Binding Energies (kcal/mol) for flexible and rigid docking of fosmidomycin, FR900098 and various (de)protonation states of DOXP using Autodock Vina. In each case the pairs of energies are for flexible and rigid docking respectively.

ligand	3au9_A	rigid	3au9_B	rigid	3aua_A	rigid	3aua_B	rigid	homo-logy	rigid	2egh_A	rigid	2egh_B	rigid	1q0l_A	rigid
fosmidomycin	-8.2	-	-8.1	-	-7.9	-	-7.9	-	-6.2	-	-7.5	-	-7.3	-	-8.2	-
FR900098	-8.2	-	-8.7	-	-8.9	-	-8.9	-	-8.0	-	-8.5	-	-8.2	-	-8.4	-
DOXP																
dp	-7.9	-7.2	-8.0	-7.3	-7.8	-7.5	-7.6	-7.5	-7.2	-6.4	-6.5	-6.8	-6.7	-6.9	-7.4	-7.1
dpni	-8.0	-7.2	-7.8	-7.6	-7.7	-7.1	-7.8	-7.5	-6.8	-6.5	-6.8	-6.6	-7.1	-7.3	-7.5	-7.3
dpnii	-8.0	-7.5	-8.0	-7.6	-7.8	-7.7	-7.8	-7.6	-6.9	-6.1	-6.9	-7.0	-6.9	-7.0	-7.4	-7.4
dpniii	-8.2	-7.5	-7.7	-7.6	-7.8	-7.7	-7.7	-7.6	-7.1	-6.1	-6.9	-7.0	-7.1	-7.0	-7.4	-7.4
dpniv	-7.9	-7.3	-7.9	-7.3	-7.9	-7.7	-7.8	-7.6	-6.7	-6.1	-6.9	-6.9	-7.0	-7.1	-7.3	-7.4
dpnni	-8.4	-7.3	-8.5	-7.3	-8.1	-7.3	-7.9	-7.7	-7.2	-6.6	-6.9	-6.5	-7.1	-7.1	-7.5	-7.0
dpnnii	-8.3	-7.5	-8.2	-7.4	-7.9	-7.6	-7.9	-7.5	-7.2	-6.4	-6.7	-6.5	-7.2	-7.2	-7.5	-7.3
dpnniii	-8.2	-7.4	-7.9	-7.3	-7.7	-7.5	-7.9	-7.6	-6.8	-6.6	-7.1	-6.6	-7.1	-7.2	-7.7	-7.3
dpnniv	-8.1	-7.6	-8.2	-7.5	-8.0	-7.6	-8.0	-7.7	-7.1	-6.7	-7.0	-6.7	-7.0	-7.2	-7.3	-7.5
dpnnni	-8.3	-7.6	-8.3	-7.5	-7.9	-7.9	-7.7	-7.9	-7.1	-6.8	-7.2	-6.5	-7.4	-7.0	-7.8	-7.5
dpnnnii	-8.4	-7.3	-8.3	-7.4	-8.0	-7.7	-7.4	-7.7	-7.0	-6.7	-7.3	-6.5	-7.0	-7.0	-7.4	-7.1
dpnnniii	-8.1	-7.3	-8.1	-7.3	-7.9	-7.3	-8.2	-7.3	-6.7	-6.6	-7.3	-6.8	-7.1	-7.4	-7.6	-7.2
dpnnniv	-8.0	-7.3	-7.9	-7.3	-7.8	-7.3	-8.2	-7.2	-6.7	-6.6	-7.2	-6.9	-7.4	-7.4	-7.8	-7.4
dpnnv	-8.0	-7.5	-8.0	-7.5	-7.8	-7.7	-8.0	-7.6	-6.8	-6.5	-6.8	-7.1	-7.0	-7.2	-7.7	-7.5
dpnnvi	-8.3	-7.4	-8.5	-7.6	-8.1	-7.5	-7.8	-7.7	-7.0	-6.2	-6.9	-6.4	-7.1	-6.9	-7.6	-7.1

©AUTHOR(S)

Table 2a. Binding Energies (kcal/mol) for flexible and rigid docking of various (de)protonation states of **3a-c** using Autodock Vina. In each case the pairs of energies are for flexible and rigid docking respectively.

ligand	3au9_A	rigid	3au9_B	rigid	3aua_A	rigid	3aua_B	rigid	Homo-logy	rigid	2egh_A	rigid	2egh_B	rigid	1q0l_A	rigid
3a	-7.4	-6.4	-7.5	-6.7	-7.0	-6.5	-7.8	-6.6	-8.6	-7.2	-7.7	-7.8	-7.9	-7.7	-7.8	-7.6
3ani	-7.4	-6.6	-7.9	-7.0	-7.8	-6.5	-7.6	-6.8	-8.3	-7.4	-7.5	-7.9	-7.5	-7.6	-7.8	-7.7
3anii	-7.4	-6.5	-7.5	-7.0	-7.2	-6.4	-7.9	-6.7	-7.8	-7.3	-7.7	-7.6	-7.7	-7.3	-7.9	-7.6
3aniii	-7.5	-6.5	-7.6	-6.9	-7.4	-6.4	-7.6	-6.7	-7.7	-7.4	-7.7	-7.6	-7.8	-7.4	-8.4	-7.6
3aniiit	-7.7	-6.5	-8.1	-6.6	-7.7	-6.4	-7.8	-6.4	-7.9	-7.3	-7.5	-7.2	-7.6	-7.2	-8.2	-7.7
3aniit	-8.0	-6.7	-7.8	-6.6	-7.5	-6.5	-7.7	-6.6	-7.8	-7.3	-7.4	-7.3	-7.6	-7.2	-8.0	-7.7
3anit	-7.9	-6.2	-7.6	-6.5	-7.7	-6.2	-7.5	-6.5	-8.0	-7.4	-7.5	-7.5	-7.6	-7.3	-8.0	-7.5
3anni	-7.2	-6.7	-8.0	-7.1	-7.3	-6.7	-7.6	-7.0	-7.7	-7.3	-7.4	-7.7	-7.6	-7.4	-8.1	-7.7
3annii	-7.3	-6.7	-8.0	-7.1	-7.9	-6.7	-7.7	-6.9	-7.8	-7.3	-7.5	-7.6	-8.0	-7.4	-7.8	-7.7
3anniii	-7.4	-6.4	-8.0	-7.0	-7.6	-6.4	-7.7	-6.7	-7.9	-7.5	-7.3	-7.1	-7.4	-7.3	-7.8	-7.5
3anniiit	-7.5	-6.4	-7.8	-6.7	-7.8	-6.4	-7.1	-6.4	-7.8	-7.5	-7.6	-7.0	-7.2	-7.1	-8.2	-7.6
3anniit	-8.1	-6.3	-8.0	-6.7	-8.0	-6.2	-7.6	-6.3	-8.0	-7.3	-7.6	-7.3	-7.7	-7.2	-7.8	-7.6
3annit	-7.3	-6.3	-7.6	-6.6	-7.8	-6.3	-7.5	-6.4	-7.8	-7.3	-7.7	-7.3	-7.7	-7.2	-8.1	-7.6
3annn	-7.9	-6.5	-7.4	-7.0	-7.4	-6.6	-7.6	-6.8	-7.9	-7.6	-7.2	-7.2	-7.3	-7.2	-8.7	-7.6
3annnt	-7.7	-6.3	-8.0	-6.7	-7.6	-6.2	-7.2	-6.5	-7.7	-7.5	-7.3	-7.0	-7.2	-7.1	-8.0	-7.4
3at	-8.2	-6.5	-8.1	-6.7	-8.0	-6.5	-7.7	-6.6	-7.5	-7.1	-7.5	-7.6	-7.9	-7.3	-8.1	-7.7
3b	-7.1	-6.2	-8.3	-6.1	-7.8	-6.5	-7.9	-6.8	-8.2	-7.6	-7.8	-7.3	-7.9	-7.7	-8.4	-7.5
3bni	-8.7	-5.4	-8.7	-6.0	-7.3	-5.4	-7.6	-5.8	-8.2	-7.6	-8.3	-7.9	-8.3	-7.4	-8.4	-7.4
3bnii	-7.3	-6.5	-8.1	-6.1	-7.7	-6.6	-8.2	-6.8	-8.2	-7.6	-8.0	-7.6	-8.1	-7.7	-8.5	-7.8
3bniii	-7.5	-6.3	-7.9	-6.3	-8.0	-6.6	-8.0	-6.8	-8.2	-7.6	-7.9	-7.6	-8.1	-7.6	-8.3	-7.8
3bniiit	-8.6	-7.0	-8.1	-7.2	-8.3	-7.0	-8.3	-7.1	-8.8	-7.7	-8.1	-7.7	-8.4	-7.6	-8.6	-7.9
3bniit	-8.6	-7.0	-8.6	-7.2	-8.3	-7.1	-8.3	-7.2	-8.2	-7.6	-8.2	-7.8	-8.1	-7.7	-8.7	-7.9
3bnit	-8.3	-6.7	-8.1	-6.9	-8.2	-6.7	-8.0	-7.1	-8.0	-7.6	-8.0	-7.8	-8.3	-7.7	-8.6	-7.7
3bnni	-7.3	-6.1	-8.3	-6.1	-8.3	-6.4	-8.3	-6.5	-8.3	-7.9	-8.1	-7.8	-8.2	-7.6	-8.6	-8.0

©**AUTHOR(S)**

Table 2b. Binding Energies (kcal/mol) for flexible and rigid docking of various (de)protonation states of **3a-c** using Autodock Vina. In each case the pairs of energies are for flexible and rigid docking respectively.

ligand	3au9_A	rigid	3au9_B	rigid	3aua_A	rigid	3aua_B	rigid	Homo-logy	rigid	2egh_A	rigid	2egh_B	rigid	1q0l_A	rigid
3bnnii	-7.4	-6.1	-8.2	-6.1	-8.2	-6.4	-8.1	-6.6	-8.3	-7.8	-8.0	-7.8	-8.2	-7.5	-8.6	-8.0
3bnniii	-7.3	-6.3	-8.5	-6.4	-7.5	-6.5	-7.6	-6.8	-8.3	-7.7	-8.1	-7.3	-7.7	-7.8	-8.7	-7.7
3bnniiit	-7.9	-6.9	-7.9	-7.0	-8.3	-6.9	-7.8	-7.0	-8.2	-7.6	-8.0	-7.5	-8.1	-7.6	-8.9	-7.9
3bnniit	-8.4	-6.7	-8.5	-6.8	-8.2	-6.9	-7.9	-7.0	-8.2	-7.7	-8.2	-7.7	-8.4	-7.6	-8.7	-7.8
3bnnit	-8.4	-6.6	-8.6	-6.8	-8.3	-6.7	-7.9	-6.9	-8.2	-7.7	-8.2	-7.7	-8.3	-7.6	-8.6	-7.8
3bnnn	-7.4	-6.0	-8.0	-6.1	-7.9	-6.2	-7.8	-6.5	-8.2	-7.9	-7.8	-7.6	-7.6	-7.7	-8.8	-7.8
3bnnnt	-8.1	-6.5	-8.2	-6.9	-8.2	-6.6	-7.9	-6.8	-8.4	-7.6	-7.8	-7.4	-7.9	-7.6	-8.7	-7.8
3bt	-8.6	-6.9	-8.6	-7.1	-8.2	-7.0	-8.1	-7.2	-8.1	-7.7	-8.1	-8.0	-8.1	-7.9	-8.7	-7.9
3c	-4.5	-4.9	-6.7	-5.1	-4.9	-5.0	-6.2	-5.0	-9.0	-8.6	-8.9	-8.4	-9.6	-7.8	-8.9	-5.7
3cni	-4.2	-4.9	-7.3	-5.3	-4.7	-5.0	-7.0	-4.9	-9.2	-8.7	-8.5	-7.9	-9.1	-8.0	-8.9	-5.4
3cnii	-4.7	-4.9	-7.0	-4.9	-6.5	-4.8	-6.7	-4.9	-8.6	-8.5	-9.1	-8.4	-8.8	-8.0	-9.0	-5.6
3cniii	-4.6	-4.9	-7.0	-5.0	-4.8	-4.8	-6.6	-5.0	-8.8	-8.6	-9.4	-8.3	-9.5	-7.7	-8.8	-5.3
3cniiit	-4.6	-5.1	-7.1	-5.2	-4.5	-5.0	-5.1	-5.3	-8.8	-8.5	-8.5	-7.6	-8.8	-7.8	-7.3	-5.4
3cniit	-4.5	-5.0	-7.0	-5.1	-4.9	-5.0	-6.7	-5.2	-8.8	-8.4	-8.4	-7.5	-8.7	-7.7	-7.4	-5.4
3cnit	-4.4	-4.7	-6.9	-4.8	-4.5	-4.7	-6.9	-4.8	-8.9	-8.4	-9.2	-7.9	-9.3	-8.1	-8.0	-5.4
3cnni	-4.4	-5.0	-7.1	-5.1	-6.7	-4.9	-6.7	-5.0	-9.0	-8.8	-8.8	-7.8	-8.7	-8.0	-9.1	-5.6
3cnnii	-4.8	-4.9	-7.7	-4.9	-6.5	-4.8	-7.1	-5.0	-8.8	-8.6	-9.1	-7.8	-9.0	-8.1	-9.3	-5.5
3cnniii	-4.0	-4.6	-6.7	-4.7	-6.5	-4.6	-6.5	-4.8	-8.9	-8.5	-9.1	-7.9	-9.0	-7.3	-8.9	-5.0
3cnniiit	-4.6	-4.8	-6.7	-4.9	-4.5	-4.8	-6.7	-5.1	-8.8	-8.1	-8.1	-7.2	-8.6	-7.2	-7.8	-5.0
3cnniit	-4.4	-4.8	-4.4	-4.9	-6.5	-4.7	-6.8	-5.0	-8.9	-8.3	-8.8	-7.8	-9.0	-7.9	-8.3	-5.6
3cnnit	-4.7	-4.9	-4.8	-5.0	-4.3	-4.8	-6.7	-4.9	-8.9	-8.4	-8.6	-7.7	-8.8	-7.8	-8.4	-5.5
3cnnn	-4.1	-4.7	-6.4	-4.9	-4.5	-4.7	-6.8	-4.9	-8.9	-8.6	-8.5	-7.6	-9.1	-7.3	-9.1	-5.2
3cnnnt	-4.5	-4.5	-4.6	-4.7	-4.5	-4.6	-6.7	-4.8	-9.1	-8.3	-8.7	-7.2	-8.5	-7.7	-7.7	-5.0
3ct	-5.0	-5.0	-7.0	-5.1	-4.3	-5.1	-5.0	-5.2	-8.4	-8.4	-8.8	-7.5	-8.8	-7.9	-7.9	-5.3

©**AUTHOR(S)**

Table 3. Binding Energies (kcal/mol) for flexible and rigid docking of various (de)protonation states of **9a-c, 10a-c** and **11a-c** using Autodock Vina. In each case the pairs of energies are for flexible and rigid docking respectively.

ligand	3au9_A	rigid	3au9_B	rigid	3aua_A	rigid	3aua_B	rigid	Homology	rigid	2eghA	rigid	2eghB	rigid	1q0l_A	rigid
9a	-6.4	-5.5	-7.0	-6.0	-7.3	-5.4	-7.6	-6.2	-7.8	-7.1	-6.6	-6.7	-7.3	-7.0	-7.2	-6.5
9b	-6.4	-4.4	-7.2	-5.1	-7.4	-5.0	-7.1	-5.8	-8.0	-7.3	-6.9	-7.1	-7.6	-7.4	-7.5	-5.0
9c	-3.8	-4.7	-6.4	-4.8	-5.7	-4.7	-6.3	-4.9	-7.8	-7.4	-7.7	-6.6	-7.8	-6.6	-7.3	-4.6
10a	-7.3	-6.6	-7.4	-6.9	-7.5	-6.2	-7.3	-6.4	-7.7	-6.7	-7.1	-7.0	-7.0	-6.9	-7.4	-7.2
10ani	-7.5	-6.9	-7.6	-7.0	-7.5	-6.6	-7.6	-6.5	-7.6	-6.8	-7.1	-6.8	-7.2	-6.7	-7.6	-7.4
10anii	-7.4	-6.9	-7.6	-7.0	-7.6	-6.6	-7.7	-6.5	-7.2	-6.8	-7.4	-6.9	-7.5	-6.8	-7.6	-7.4
10ann	-8.1	-6.8	-8.1	-7.0	-7.5	-6.6	-7.5	-6.6	-7.4	-6.8	-6.8	-6.6	-6.8	-6.7	-7.6	-7.3
10b	-7.5	-6.7	-7.6	-7.1	-7.7	-6.6	-7.8	-6.9	-8.4	-7.0	-7.6	-7.4	-7.5	-7.5	-8.2	-7.6
10bni	-7.3	-6.8	-8.4	-7.2	-7.9	-6.6	-7.5	-6.9	-8.0	-7.1	-7.4	-7.3	-7.6	-7.3	-7.9	-7.6
10bnii	-7.3	-6.9	-7.9	-7.3	-7.9	-6.6	-8.0	-7.0	-8.5	-7.1	-7.4	-7.1	-7.5	-7.3	-7.9	-7.7
10bnn	-8.0	-6.7	-7.9	-7.1	-7.9	-6.4	-7.9	-6.6	-7.9	-7.1	-7.2	-7.3	-7.5	-7.2	-8.1	-7.6
10c	-7.3	-4.8	-7.3	-4.7	-7.0	-4.8	-6.8	-4.9	-8.4	-8.0	-8.3	-7.8	-8.6	-8.2	-8.8	-6.1
10cni	-7.0	-4.7	-7.3	-4.7	-7.2	-4.7	-6.2	-4.8	-8.5	-8.0	-8.2	-7.8	-8.1	-8.1	-8.2	-6.1
10cnii	-6.5	-4.8	-7.0	-4.7	-7.0	-4.8	-7.1	-4.8	-8.6	-8.2	-8.3	-7.8	-8.5	-8.0	-8.8	-6.0
10cnn	-4.3	-4.6	-6.8	-4.6	-6.6	-4.6	-6.1	-4.6	-8.8	-7.8	-8.0	-7.4	-7.7	-7.8	-8.2	-5.9
11a	-7.3	-4.5	-7.4	-5.7	-6.7	-5.4	-6.9	-5.8	-7.2	-8.0	-7.2	-7.0	-7.7	-7.3	-7.1	-5.9
11an	-6.8	-4.4	-7.0	-5.5	-7.0	-5.0	-7.4	-5.7	-8.1	-7.9	-7.2	-7.0	-7.5	-7.3	-8.1	-5.3
11ant	-6.2	-4.4	-7.4	-5.0	-6.1	-4.3	-6.9	-4.7	-7.6	-7.9	-7.3	-7.2	-7.2	-7.2	-8.6	-5.1
11at	-6.2	-4.4	-7.0	-5.1	-7.2	-4.5	-7.1	-4.5	-8.0	-8.0	-7.0	-7.0	-7.4	-7.3	-7.6	-5.1
11b	-6.1	-4.8	-6.6	-4.9	-7.2	-5.0	-7.1	-5.4	-8.0	-7.9	-7.8	-7.4	-7.9	-7.5	-7.6	-5.1
11bn	-6.8	-4.5	-6.3	-4.7	-6.4	-4.5	-7.2	-4.9	-8.1	-7.8	-7.8	-7.4	-7.7	-7.4	-8.7	-5.1
11bnt	-6.6	-4.5	-6.5	-4.6	-7.1	-4.5	-6.6	-4.7	-8.1	-8.0	-7.5	-7.7	-8.1	-7.8	-7.4	-4.9
11bt	-6.2	-4.8	-6.9	-4.8	-7.4	-4.9	-6.9	-4.9	-8.4	-8.0	-7.6	-7.4	-7.8	-7.6	-8.3	-4.9
11c	-3.9	-4.5	-5.7	-4.6	-6.3	-4.5	-4.3	-4.6	-7.7	-7.7	-8.2	-7.0	-7.9	-6.6	-7.7	-4.9
11cn	-4.2	-4.7	-4.5	-4.8	-6.6	-4.6	-4.4	-4.8	-8.0	-8.0	-8.1	-7.1	-8.0	-6.6	-7.5	-5.0
11cnt	-4.2	-4.6	-6.0	-4.7	-6.0	-4.6	-4.0	-4.5	-7.9	-7.8	-8.3	-6.8	-8.5	-7.3	-6.7	-4.8
11ct	-4.4	-4.7	-5.8	-4.8	-6.2	-4.8	-4.5	-5.0	-8.1	-7.7	-8.2	-6.7	-8.3	-7.4	-7.0	-4.9

III. Saturation Transfer Difference (STD) NMR binding protocol

The STD experiment [1,2] was run on the ligands as follows: *Ec*DXR enzyme, stored in sodium phosphate buffer (pH 7.0), was freeze-dried and re-suspended in D_2O to make a final concentration of 20 μM. Each set of ligands was dissolved in the protein solution to give a final ligand concentration of 800 μM and thus a protein:ligand molar ratio of 1:40. The STD experiment was carried out using parameters optimized in a previous study in our group. [1] The saturating on-resonance and off-resonance pulses were set at frequencies of 0.73 ppm and 20

 ©AUTHOR(S)

ppm, respectively, while cycling between the on- and off-resonance phases was used to reduce the effects of changes in temperature or magnetic field homogeneity. A 3-9-19 water suppression pulse was applied at 4.7 ppm and 6000 scans were acquired. The on- and off-resonance spectra were subtracted from each other and processed using Bruker Topspin software.

[1] Mayer, M.; Meyer, B. *Angew Chem. Int. Ed*. 1999, *38*, 1784-1788.
DOI 10.1002/(SICI)1521-3773(19990614)**38**:12<1784::AID-ANIE1784>3.0.CO;2-Q.
[2] Marchioro C.; Davalli S.; Provera S.; Heller M.; Ross A.; Senn, H. *Experiments in NMR Based Screening*, in *BioNMR in Drug Research*, Zerbe O., Ed.; Wiley-VCH: Weinheim, 2003, Vol. 16, pp. 321-339.
[3] Conibear A. C., Synthesis and Evaluation of Novel Inhibitors of 1-Deoxy-D-xylulose-5-phosphate Reductoisomerase as Potential Antimalarials, MSc. Thesis, Rhodes University, Grahamstown, 2010.

 ©AUTHOR(S)

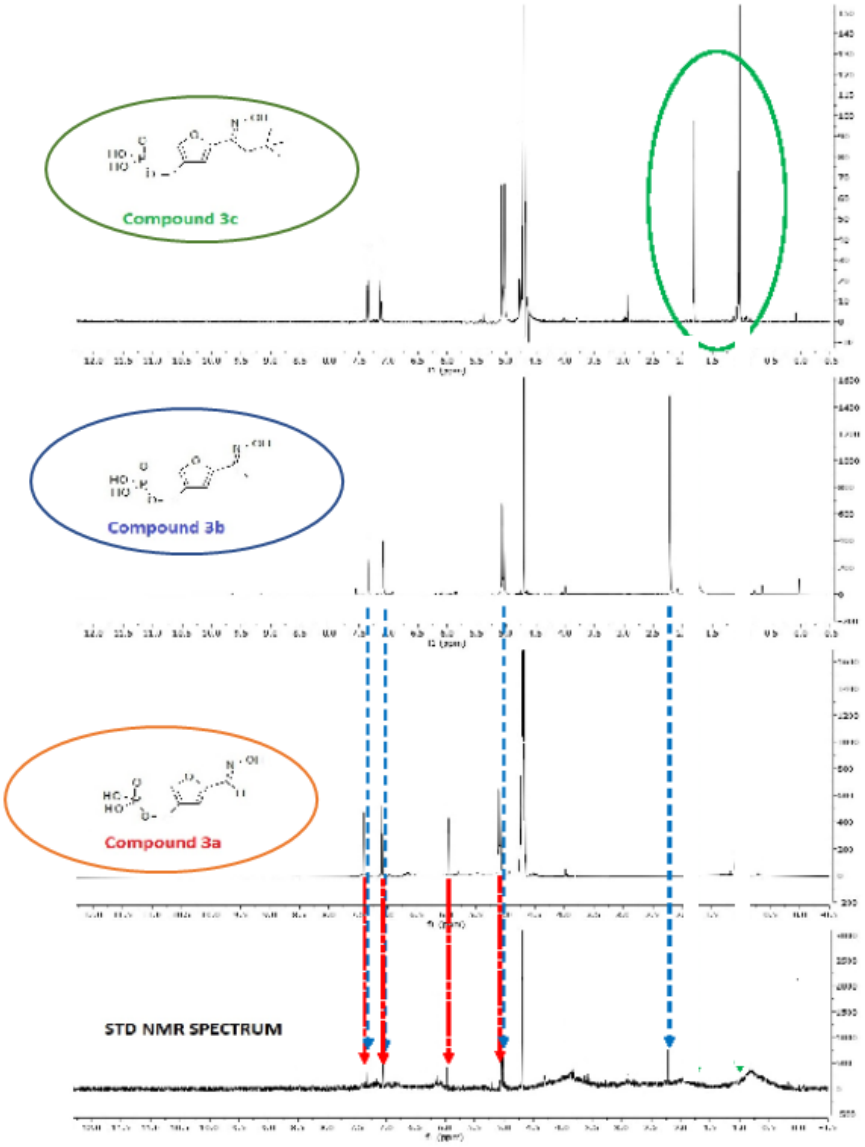


Figure 3. STD NMR correlations for binding of furan derivatives **3a** and **3b** to *Ec*DXR.

©AUTHOR(S)