

# **Synthesis and bioluminescence of electronically modified and rotationally restricted colour-shifting infraluciferins**

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*and*

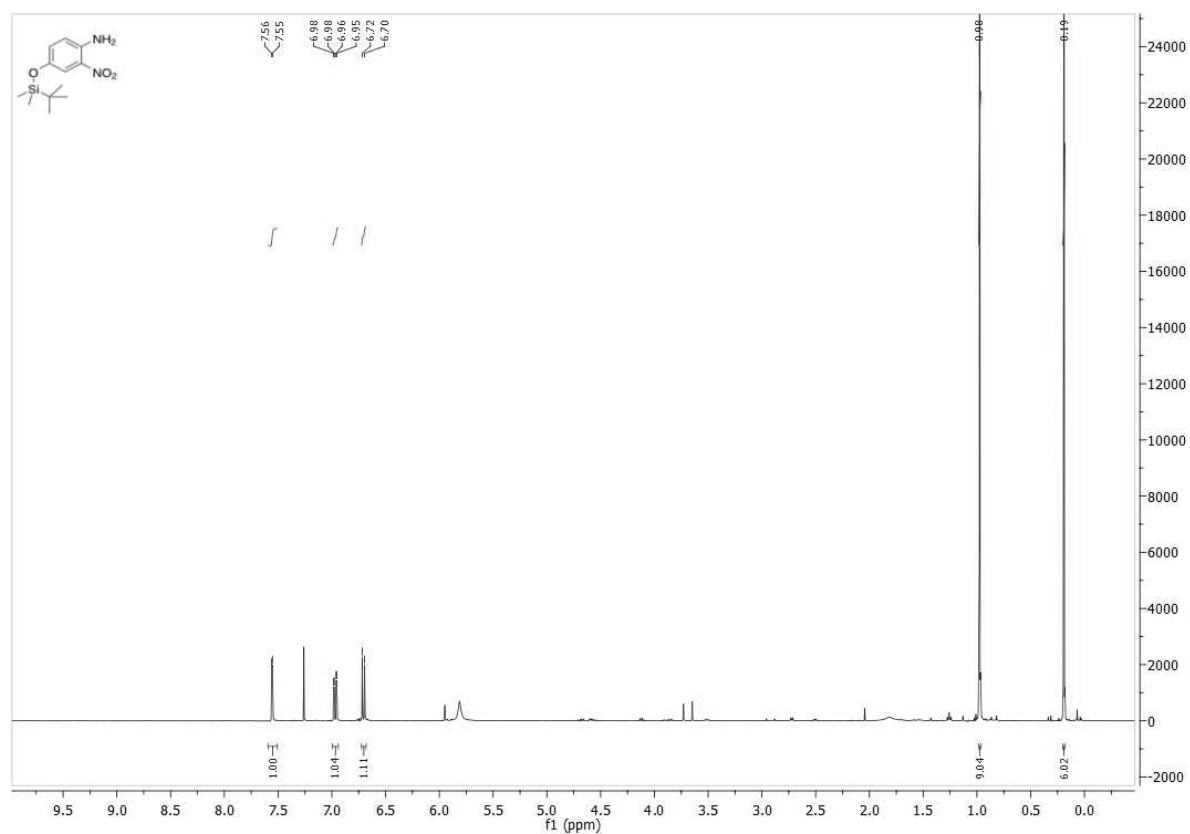
*School of Biosciences, University of Cardiff, Sir Martin Evans Building, Museum Avenue, Cardiff, CF10 3AX, UK*

## **Supplementary Information**

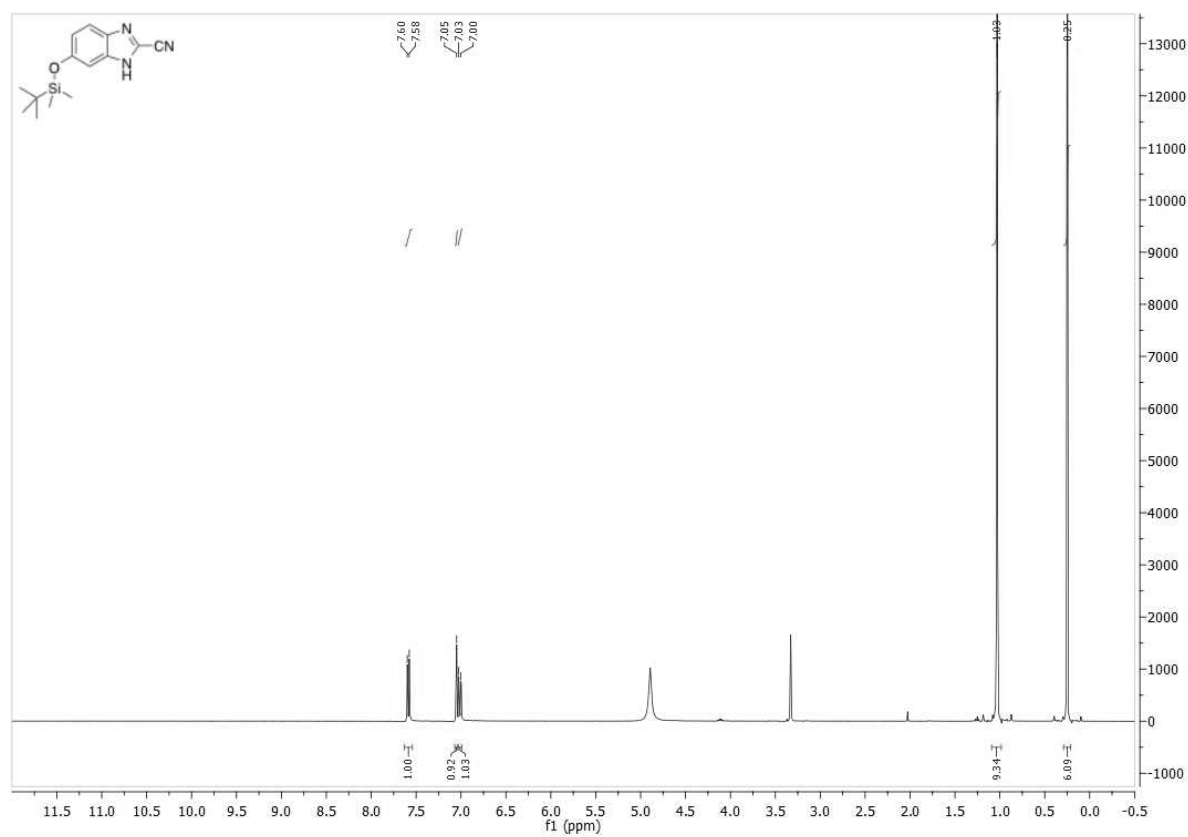
Copies of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra

Graphs of emission kinetics of enzymes with different compounds acquired in the PIO

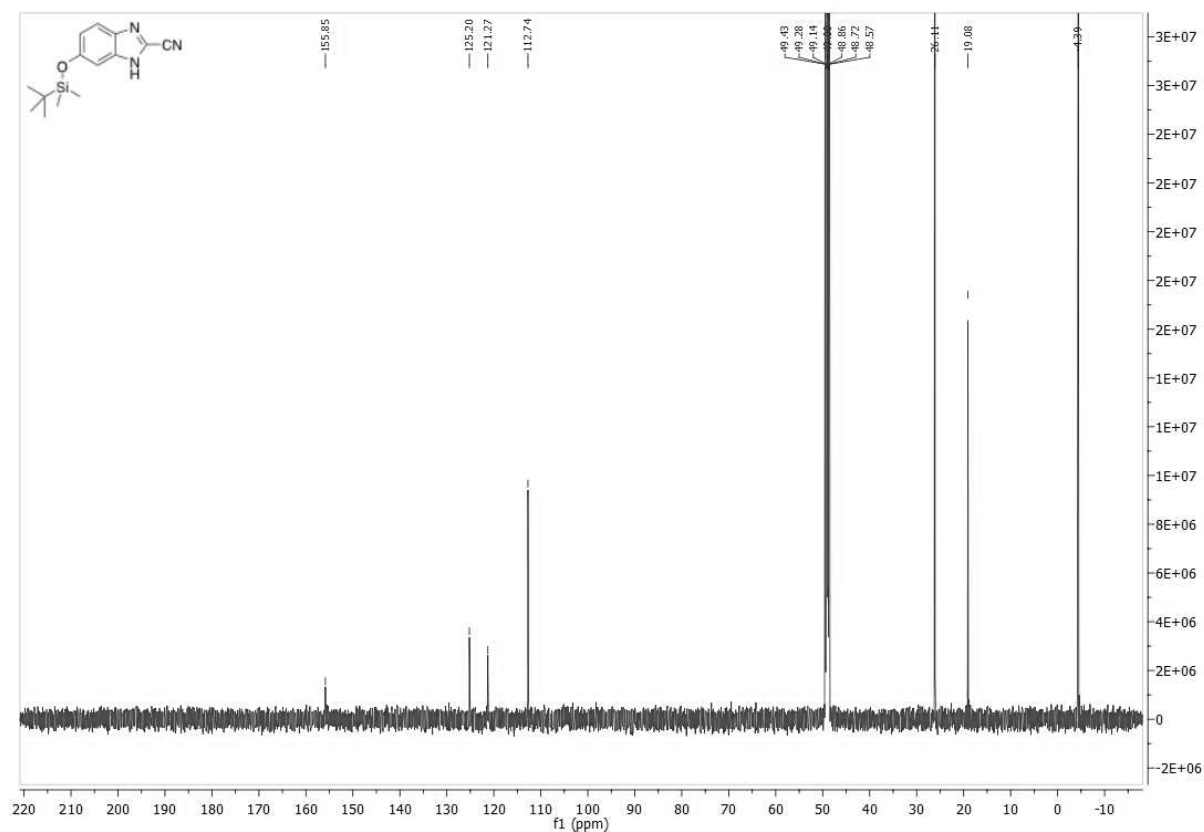
**<sup>1</sup>H – NMR: 4-((*tert*-butyldimethylsilyl)oxy)-2-nitroaniline (15)**



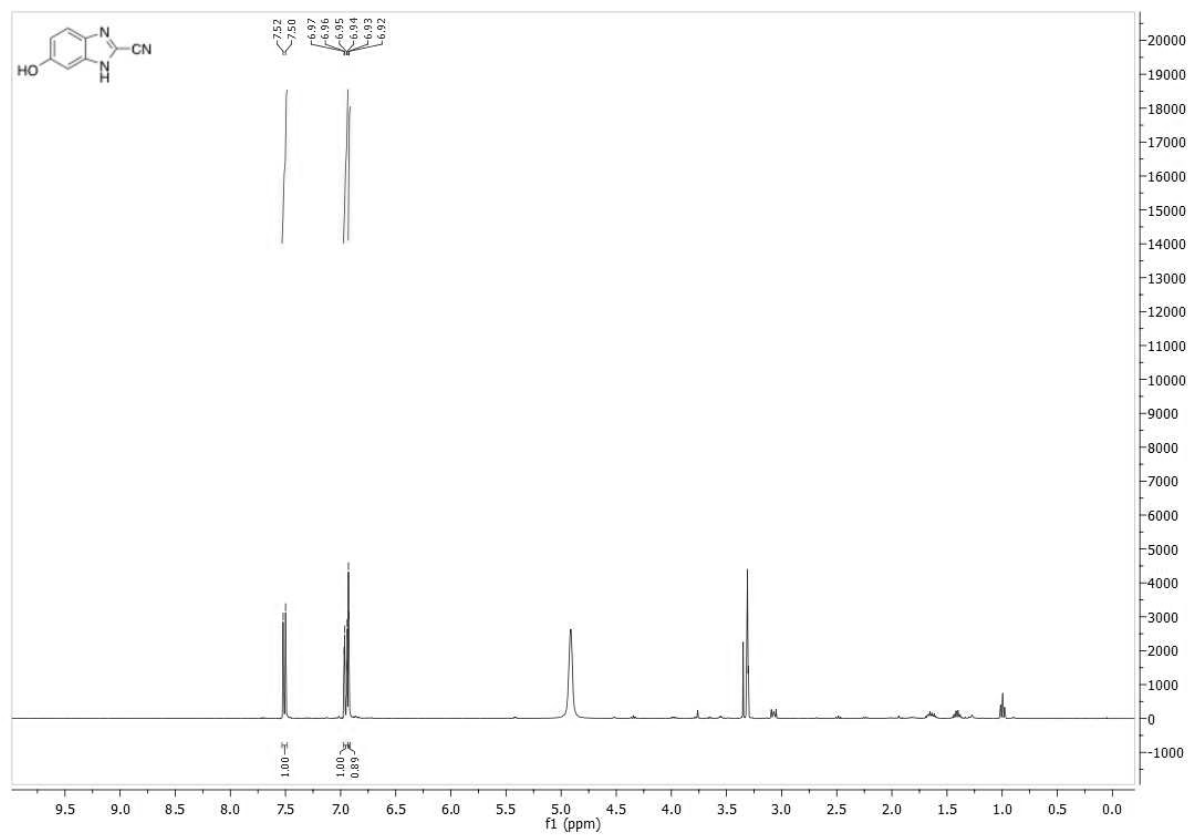
**<sup>1</sup>H – NMR: 6-((*tert*-butyldimethylsilyl)oxy)-1H-benzo[d]imidazole-2-carbonitrile (16)**



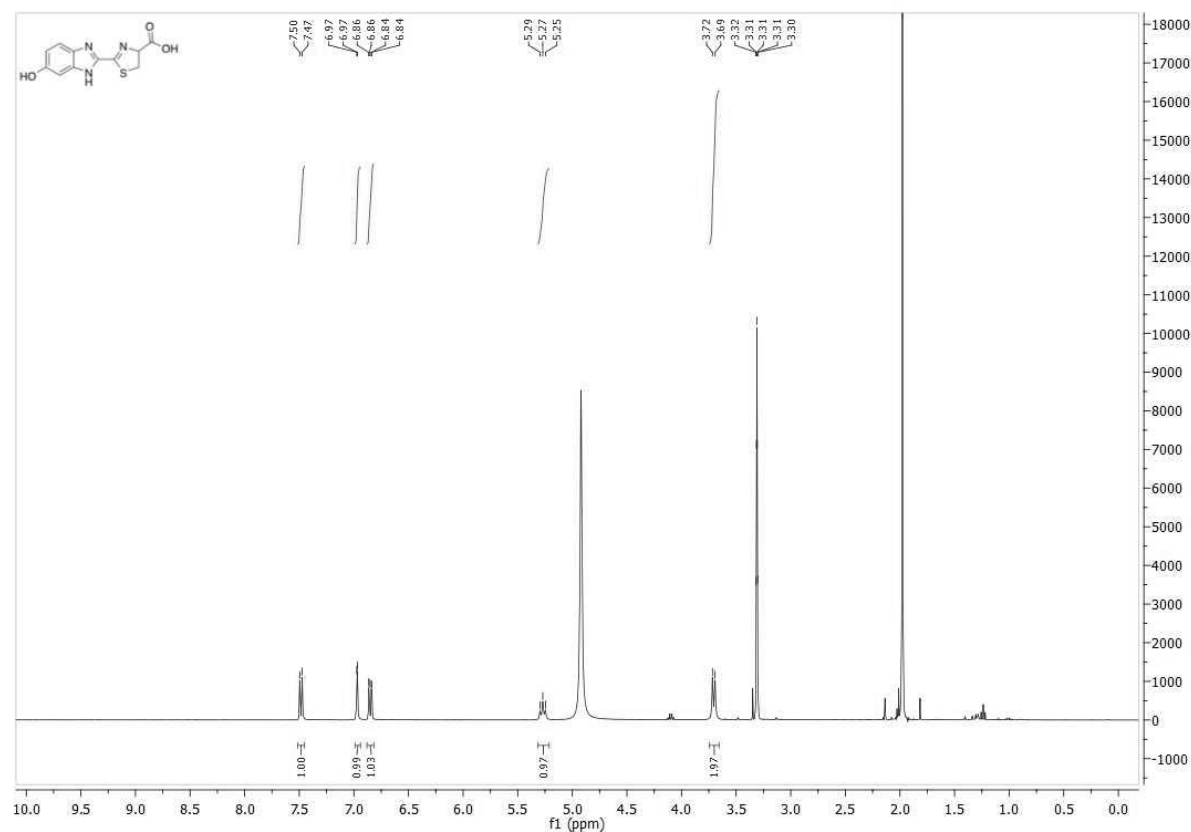
**$^{13}\text{C}$  – NMR:** 6-((tert-butyldimethylsilyl)oxy)-1H-benzo[d]imidazole-2-carbonitrile (**16**)



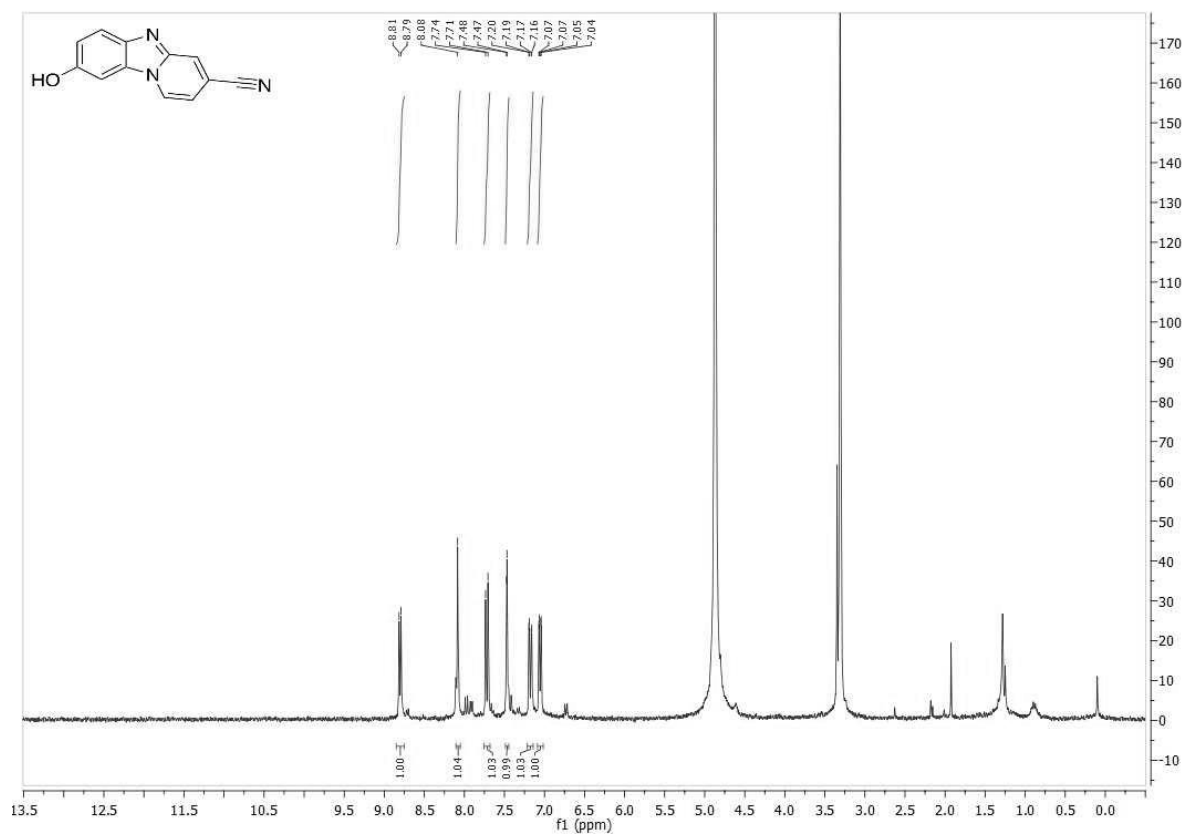
**$^1\text{H}$  – NMR:** 6-hydroxy-1H-benzo[d]imidazole-2-carbonitrile (**17**)



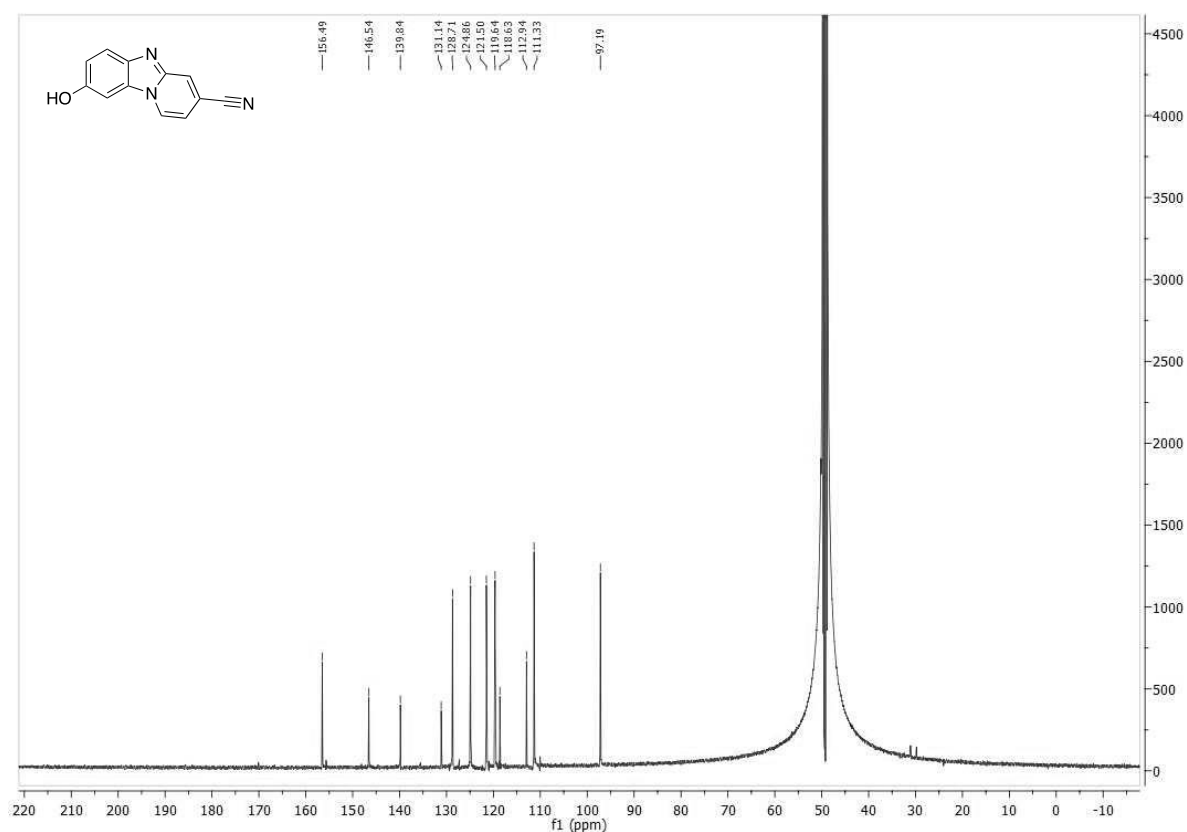
**<sup>1</sup>H – NMR: 2-(6-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid (10)**



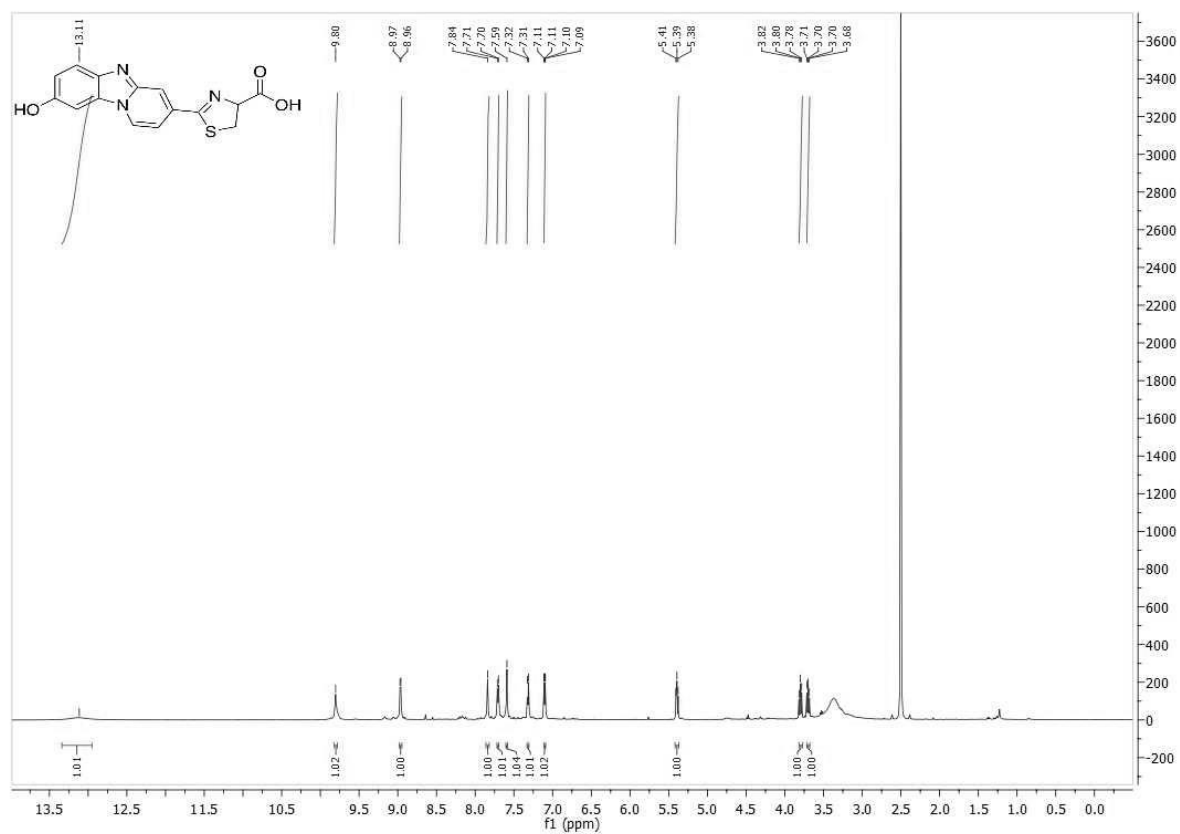
**<sup>1</sup>H – NMR: 8-hydroxybenzo[4,5]imidazo[1,2-*a*]pyridine-3-carbonitrile (20)**



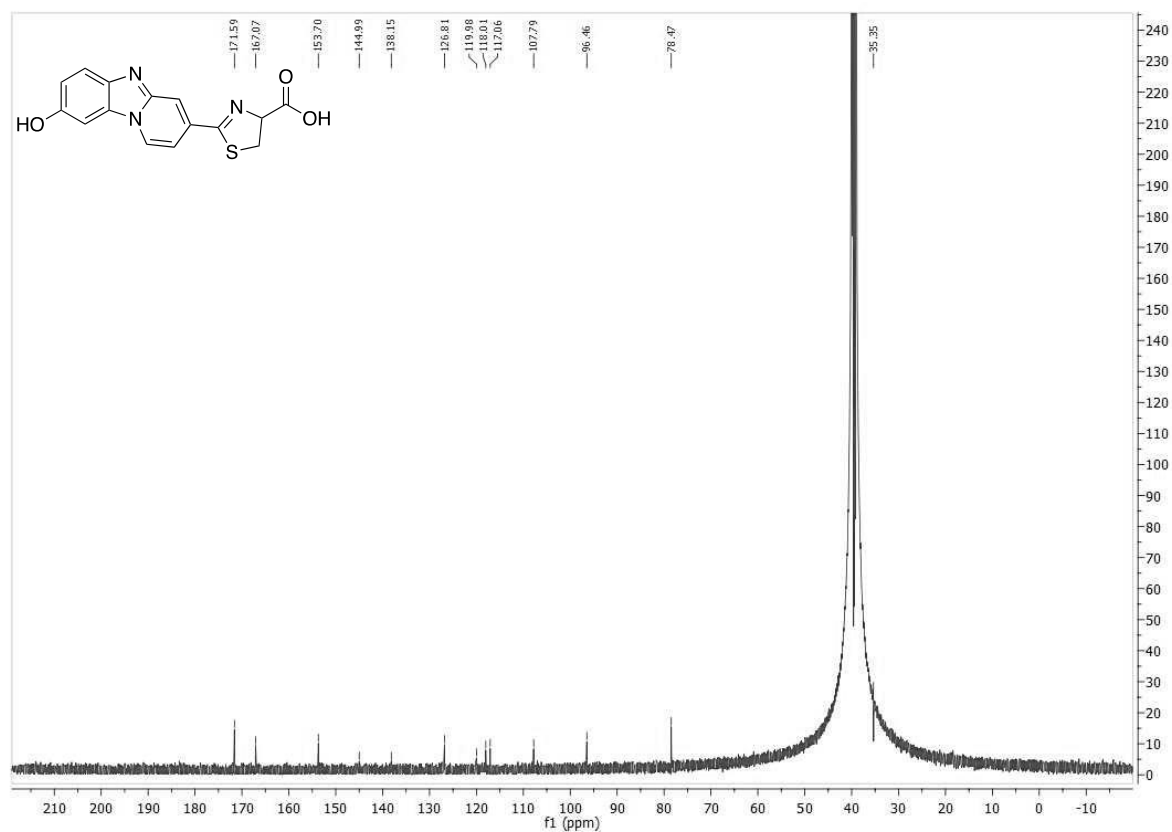
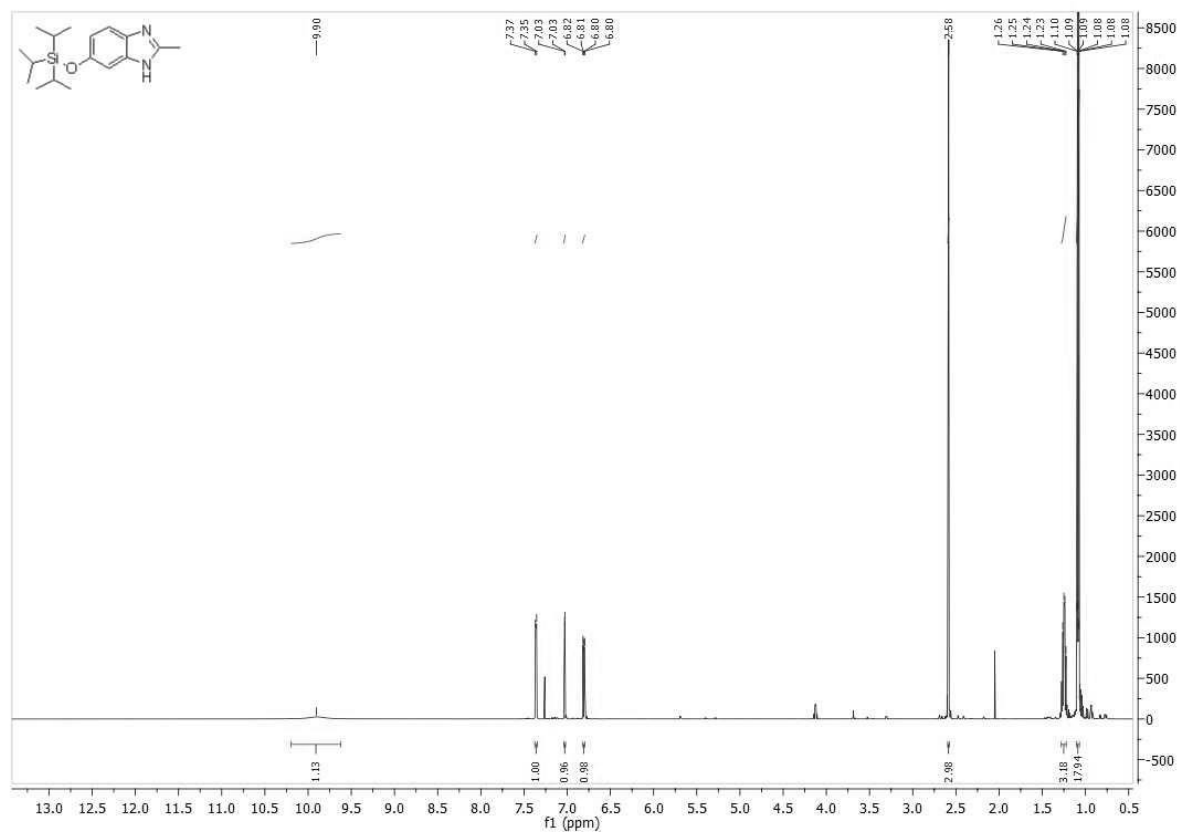
**<sup>13</sup>C-NMR:** 8-hydroxybenzo[4,5]imidazo[1,2-*a*]pyridine-3-carbonitrile (**20**)



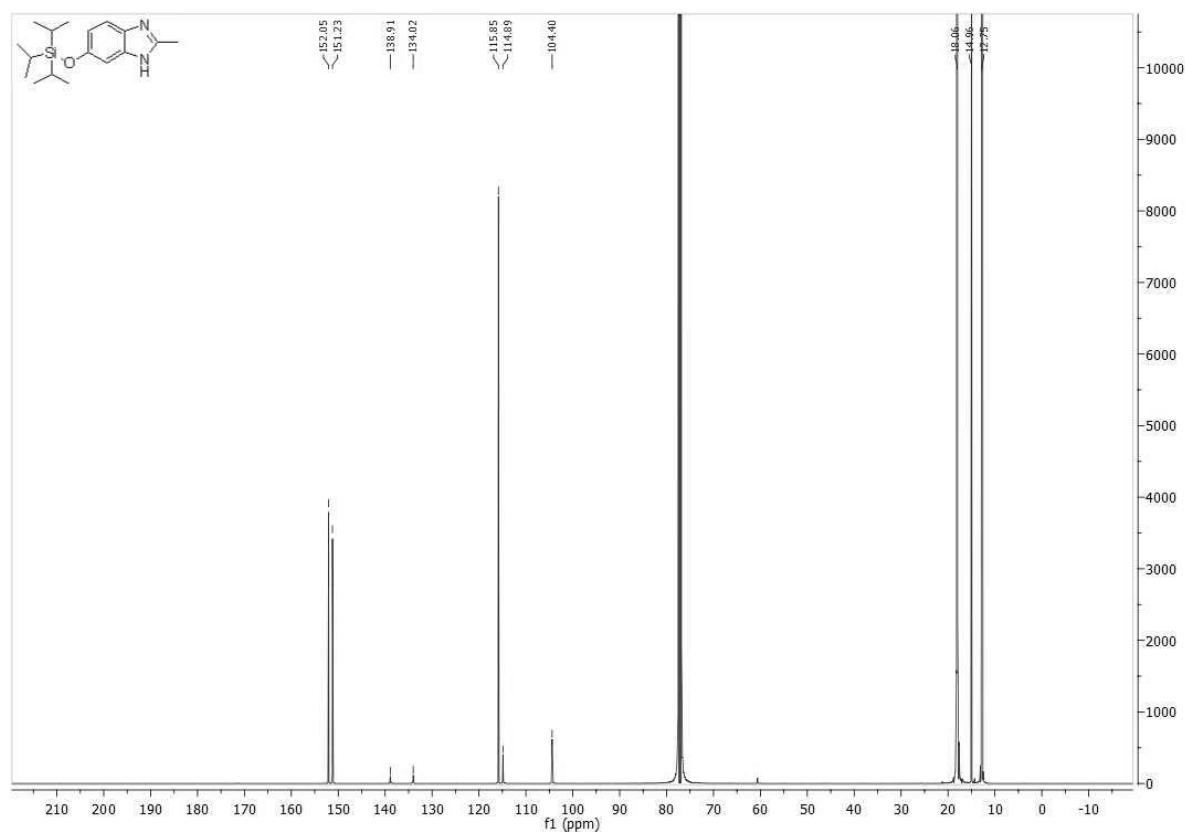
**<sup>1</sup>H – NMR:** 2-(8-hydroxybenzo[4,5]imidazo[1,2-*a*]pyridin-3-yl)-4,5-dihydrothiazole-4-carboxylic acid (**12**)



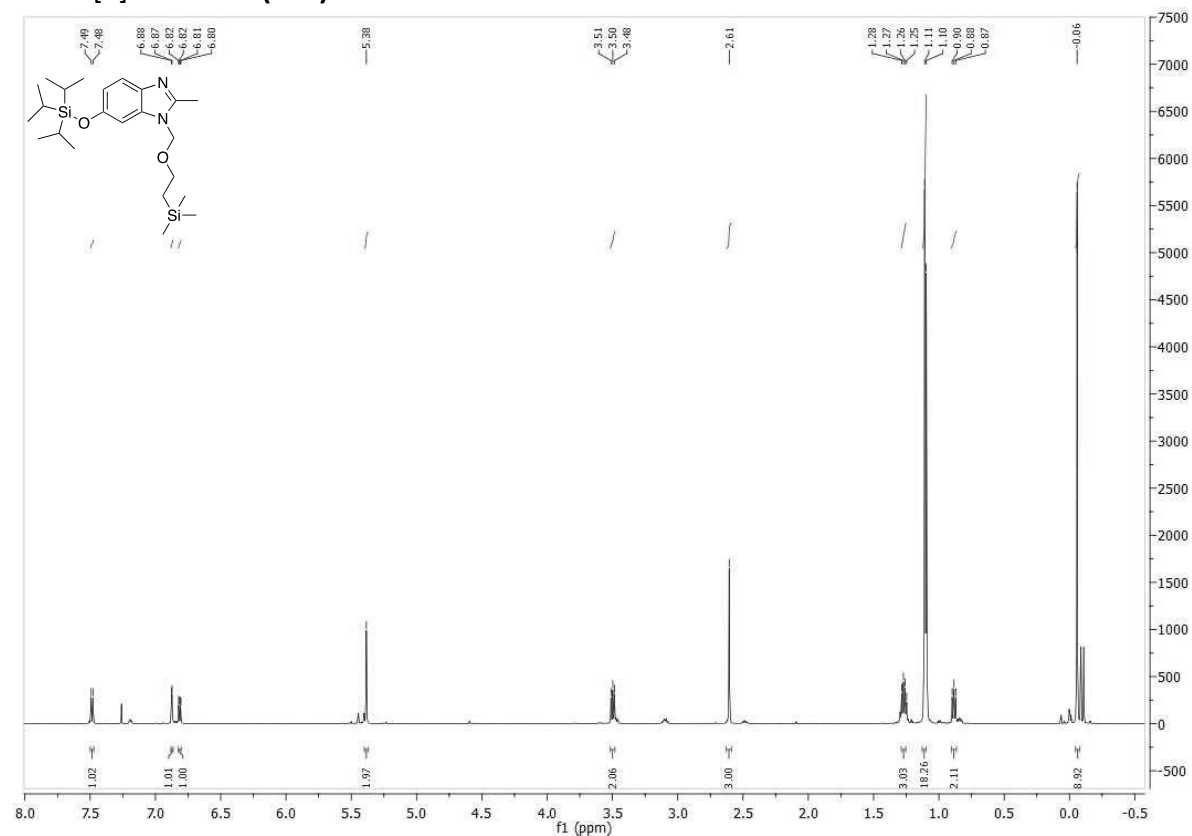
**<sup>13</sup>C-NMR:** 2-(8-hydroxybenzo[4,5]imidazo[1,2-*a*]pyridin-3-yl)-4,5-dihydrothiazole-4-carboxylic acid (**12**)

<sup>1</sup>H – NMR: 2-methyl-6-((triisopropylsilyl)oxy)-1*H*-benzo[*d*]imidazole (**22**)

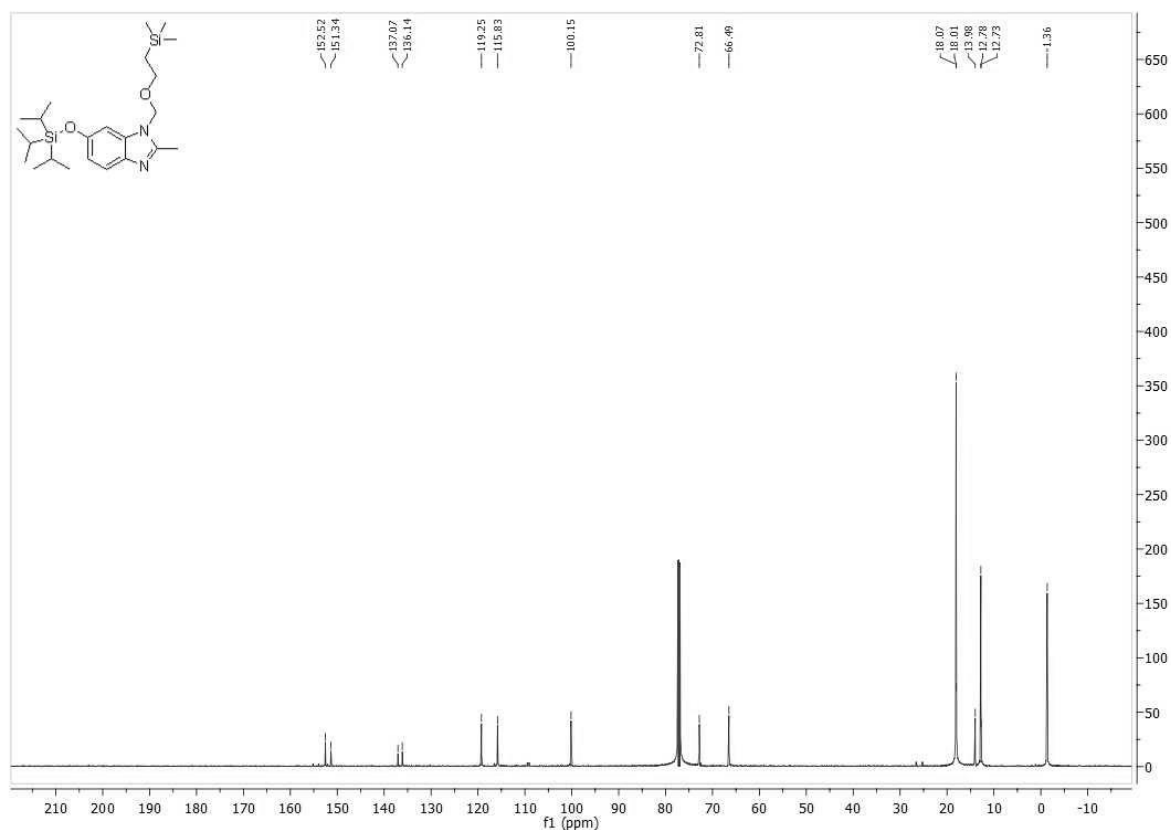
**<sup>13</sup>C-NMR: 2-methyl-6-((triisopropylsilyl)oxy)-1*H*-benzo[*d*]imidazole (22)**



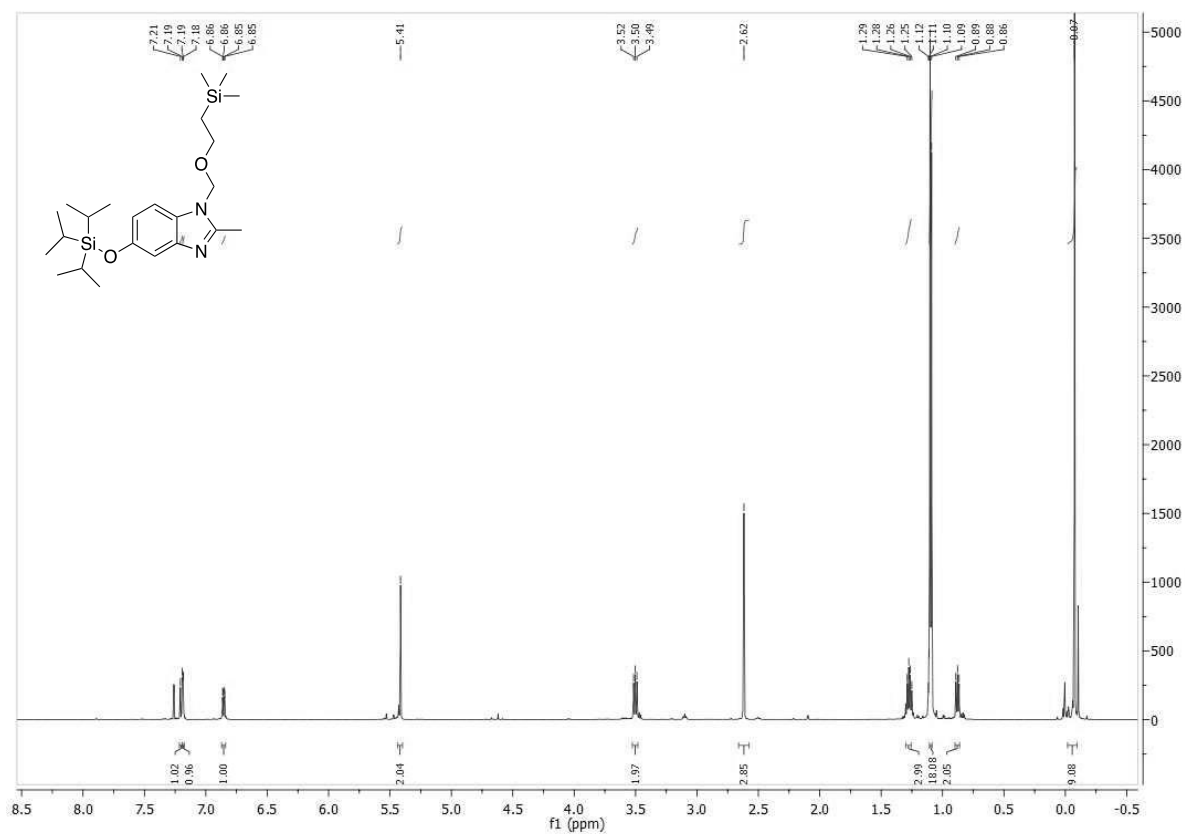
**<sup>1</sup>H – NMR: 2-methyl-6-((triisopropylsilyl)oxy)-1-((2- (trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazole (23a)**



**<sup>13</sup>C-NMR:** 2-methyl-6-((triisopropylsilyl)oxy)-1-((2- (trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazole (**23a**)

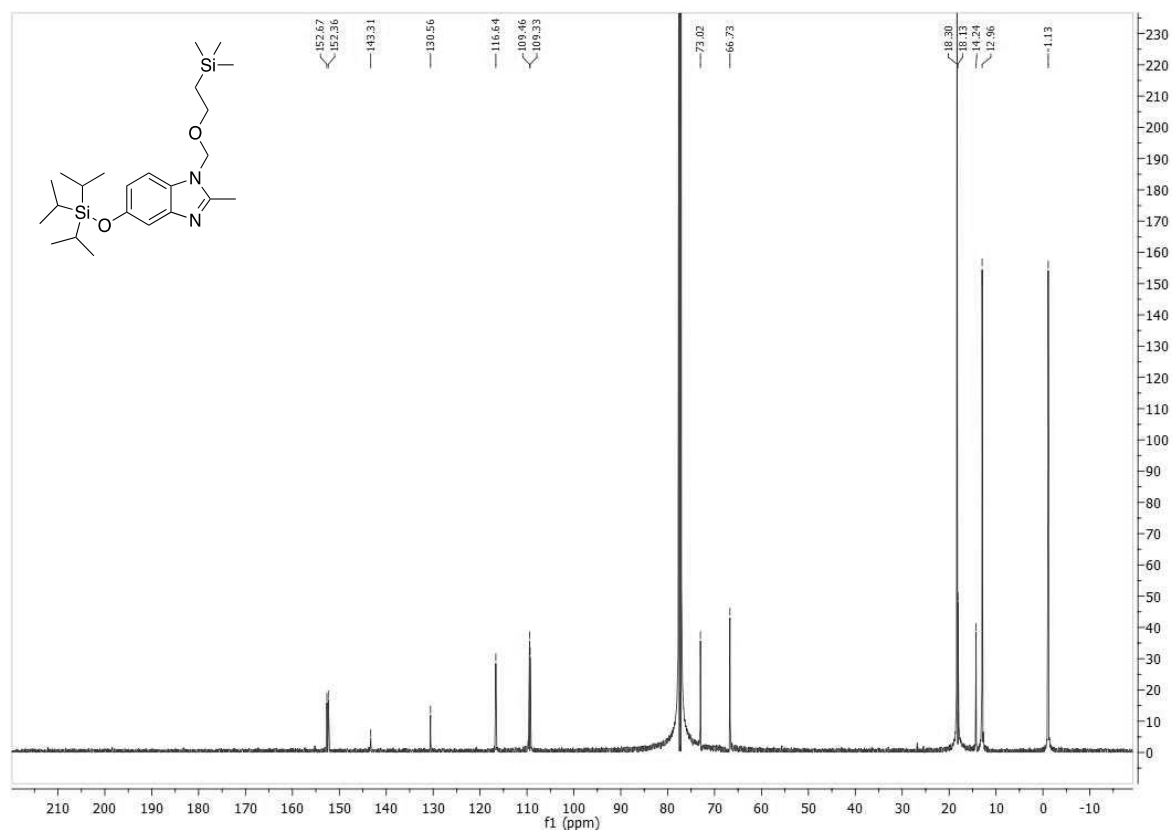


**<sup>1</sup>H – NMR:** 2-methyl-5-((triisopropylsilyl)oxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazole (**23b**)

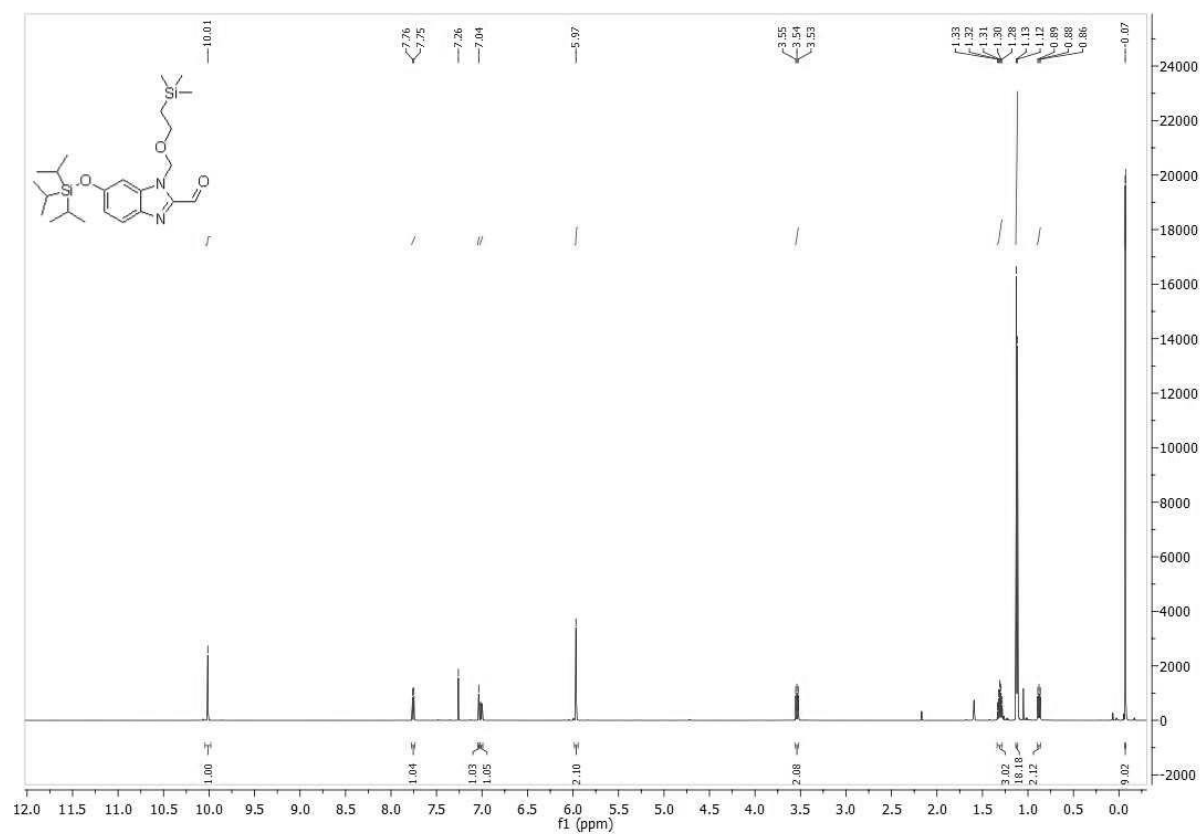




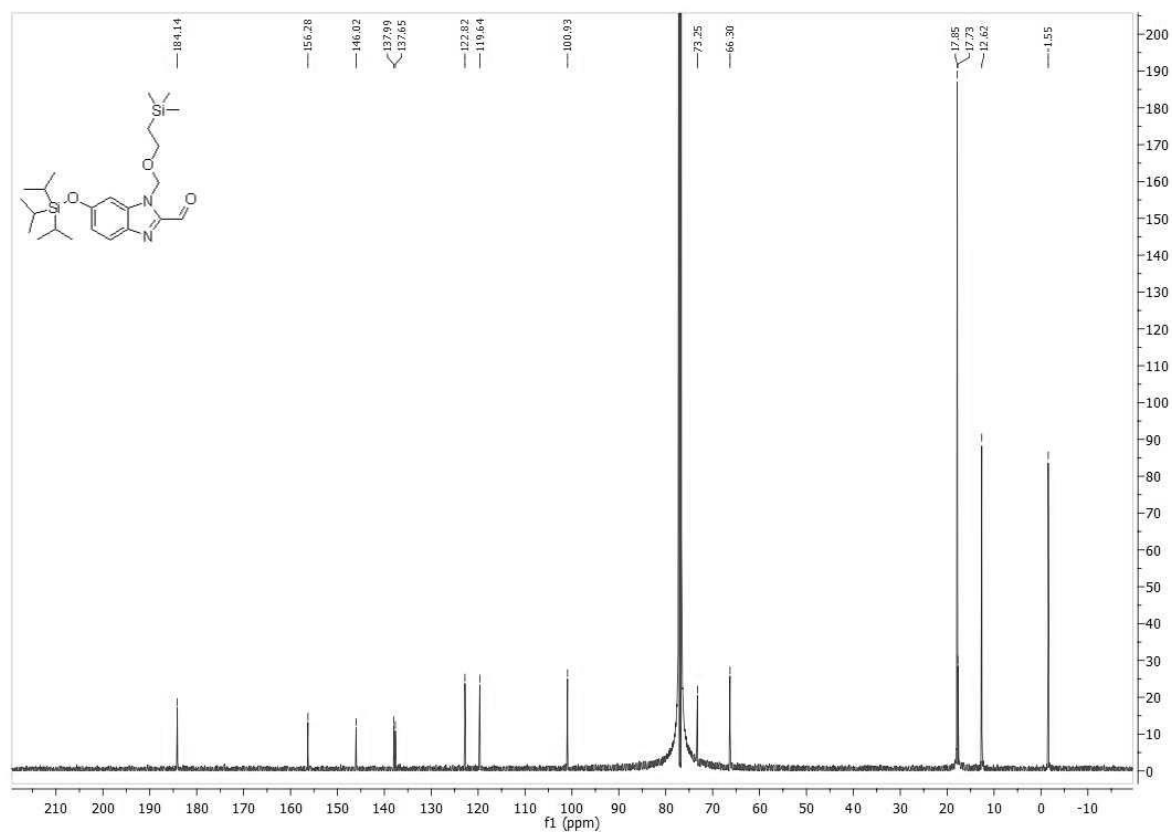
**<sup>13</sup>C-NMR:** 2-methyl-5-((triisopropylsilyl)oxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazole (**23b**)



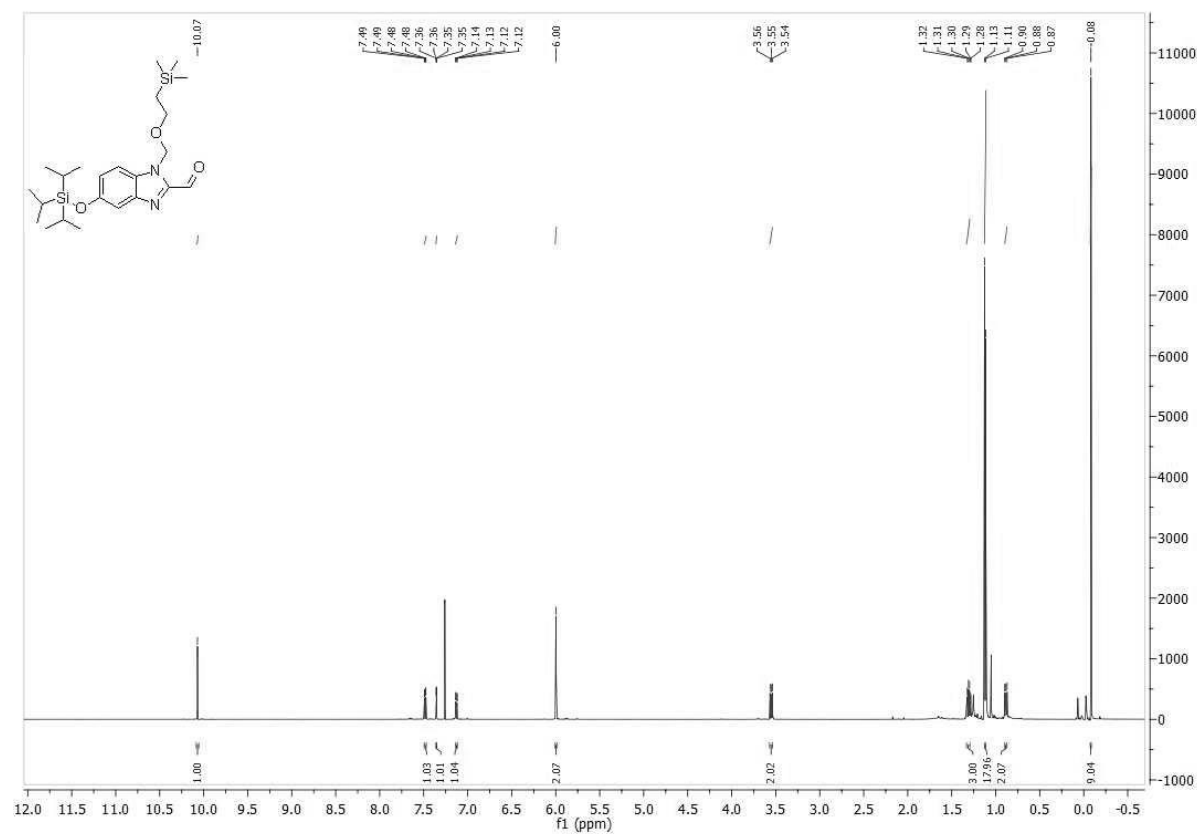
**<sup>1</sup>H – NMR:** 6-((triisopropylsilyl)oxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**24a**)



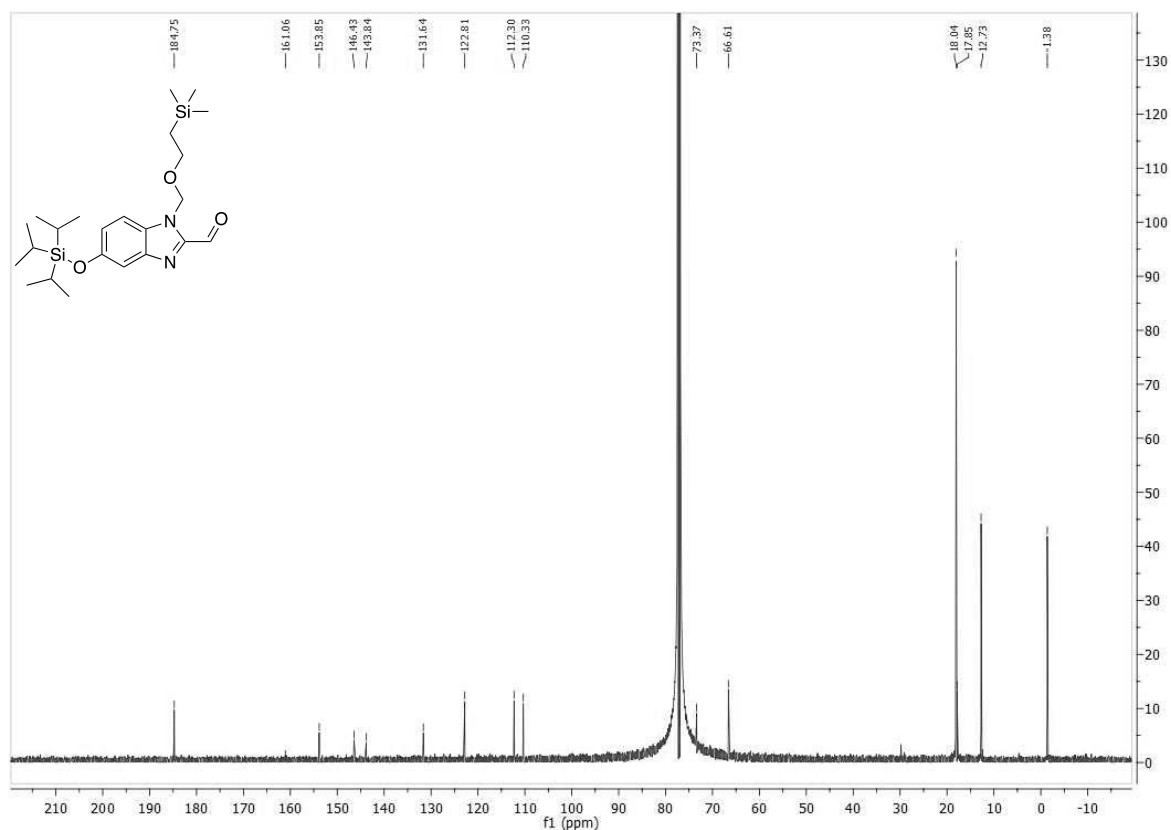
**<sup>13</sup>C-NMR:** 6-((triisopropylsilyl)oxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**24a**)



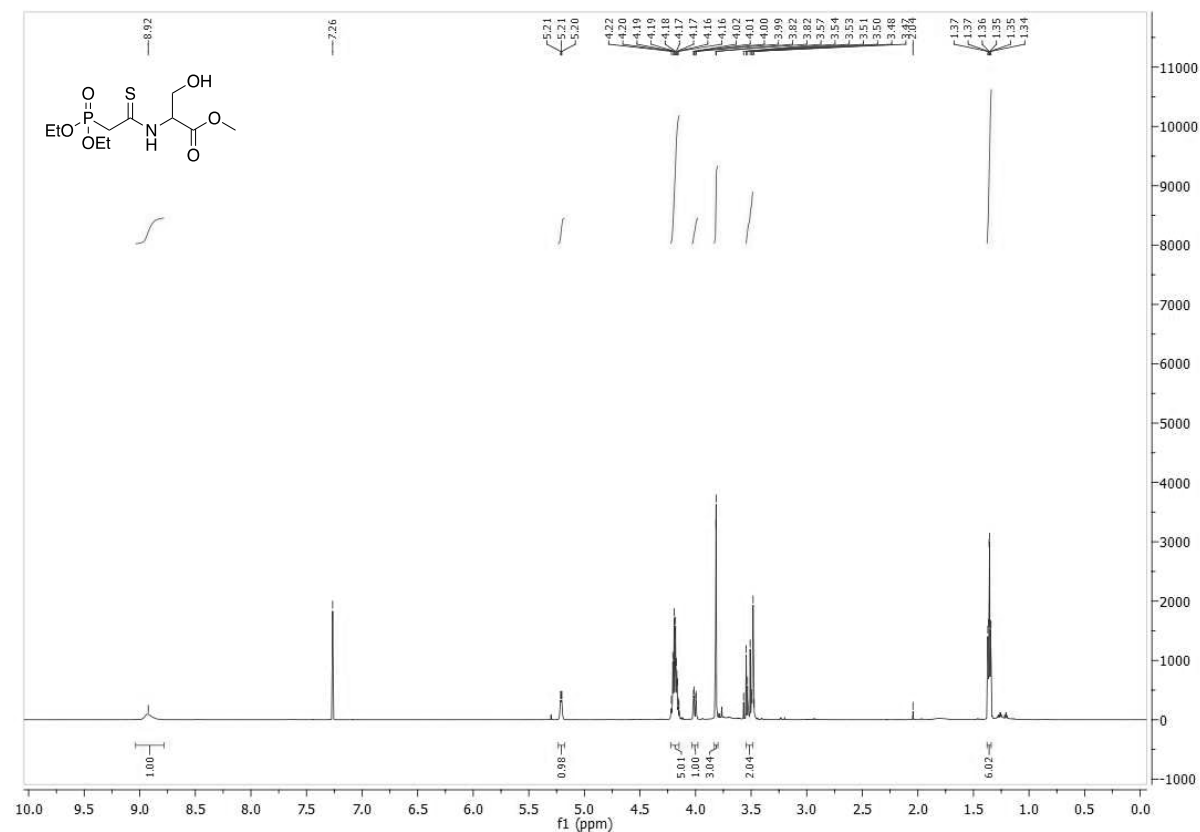
**<sup>1</sup>H – NMR:** 5-((triisopropylsilyl)oxy)-1-((2-(trimethylsilyl) ethoxy) methyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**24b**)



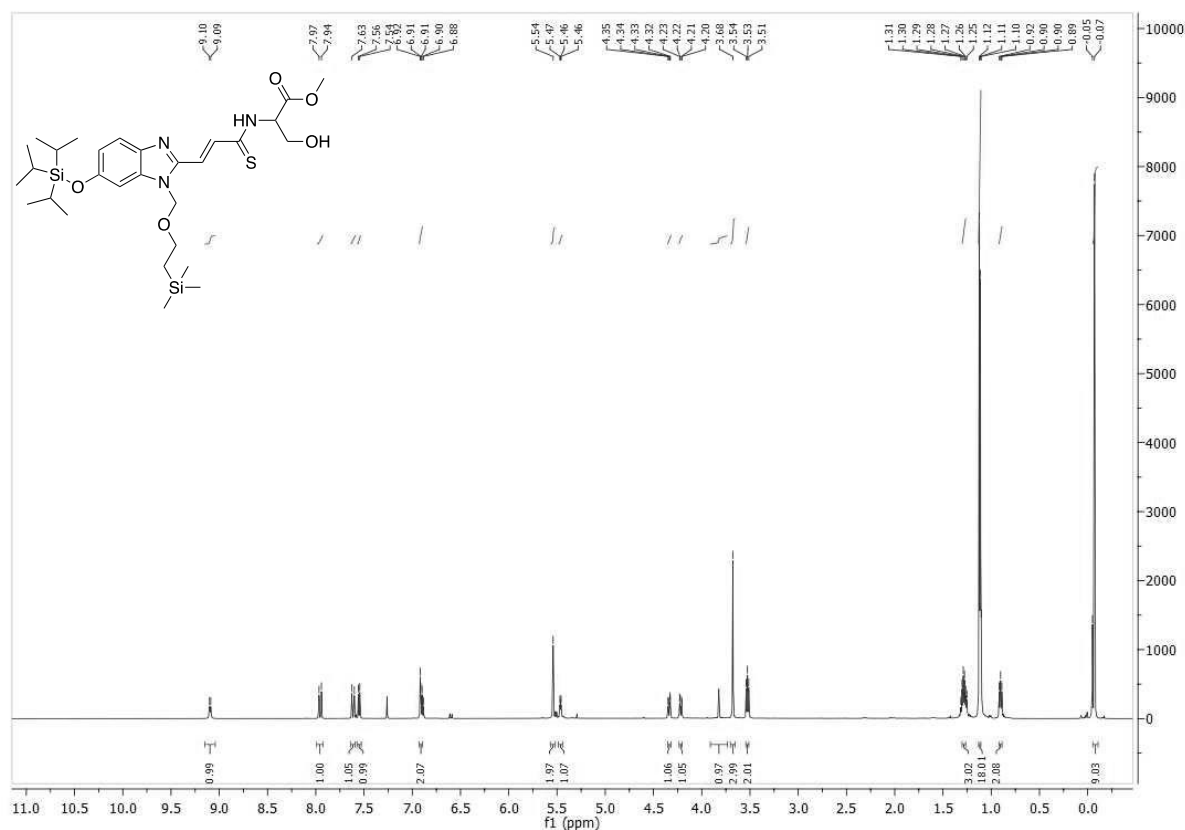
**<sup>13</sup>C-NMR:** 5-((triisopropylsilyl)oxy)-1-((2-(trimethylsilyl) ethoxy) methyl)-1H-benzo[d]imidazole-2-carbaldehyde (**24b**)



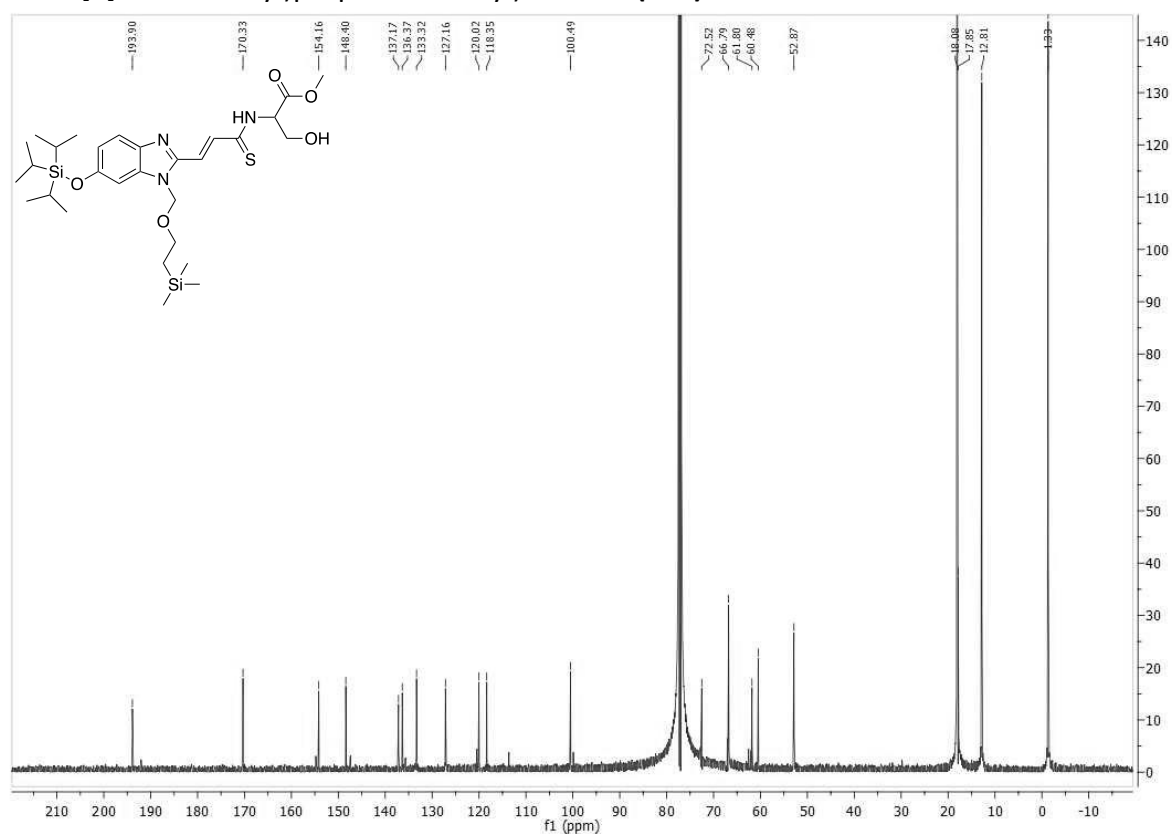
**<sup>1</sup>H – NMR:** methyl (2-(diethoxyphosphoryl)ethanethioyl)serinate (**25**)



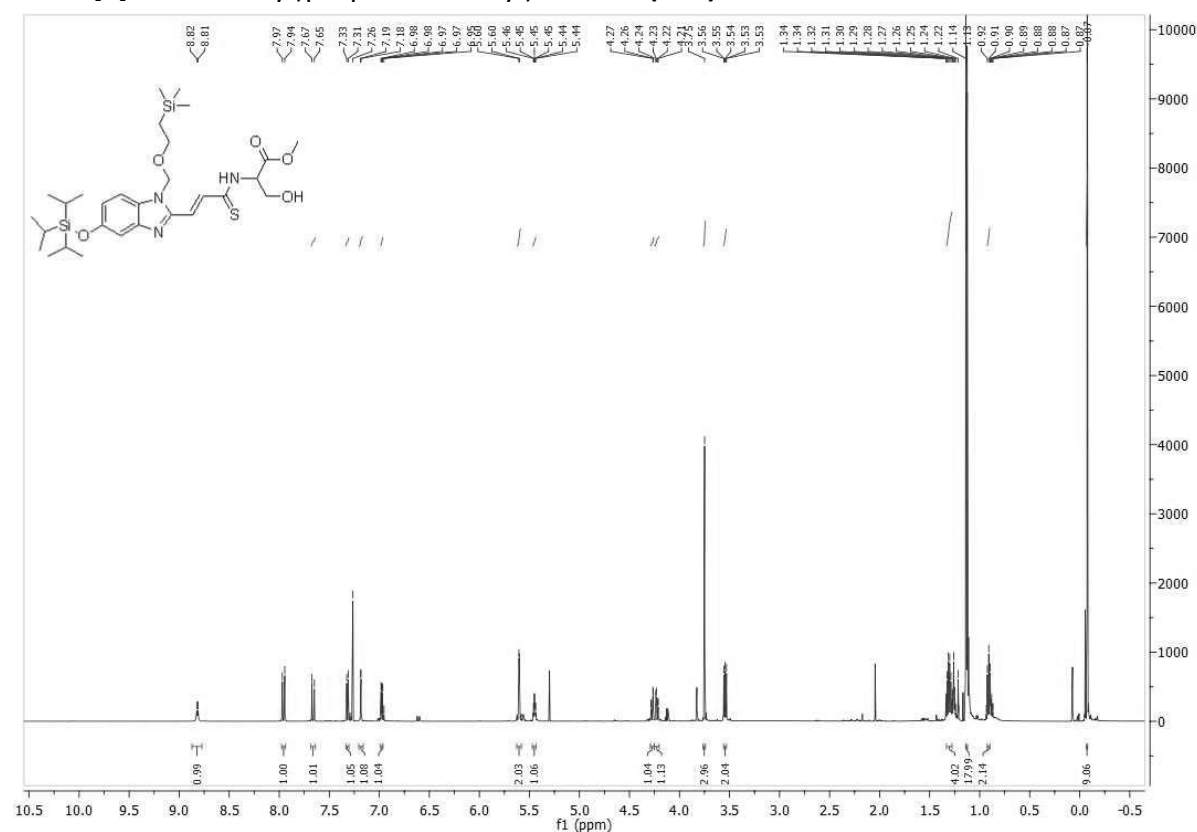
**<sup>1</sup>H – NMR:** methyl (*E*)-(3-(6-((triisopropylsilyl)oxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)prop-2-enethioyl)serinate (**26a**)



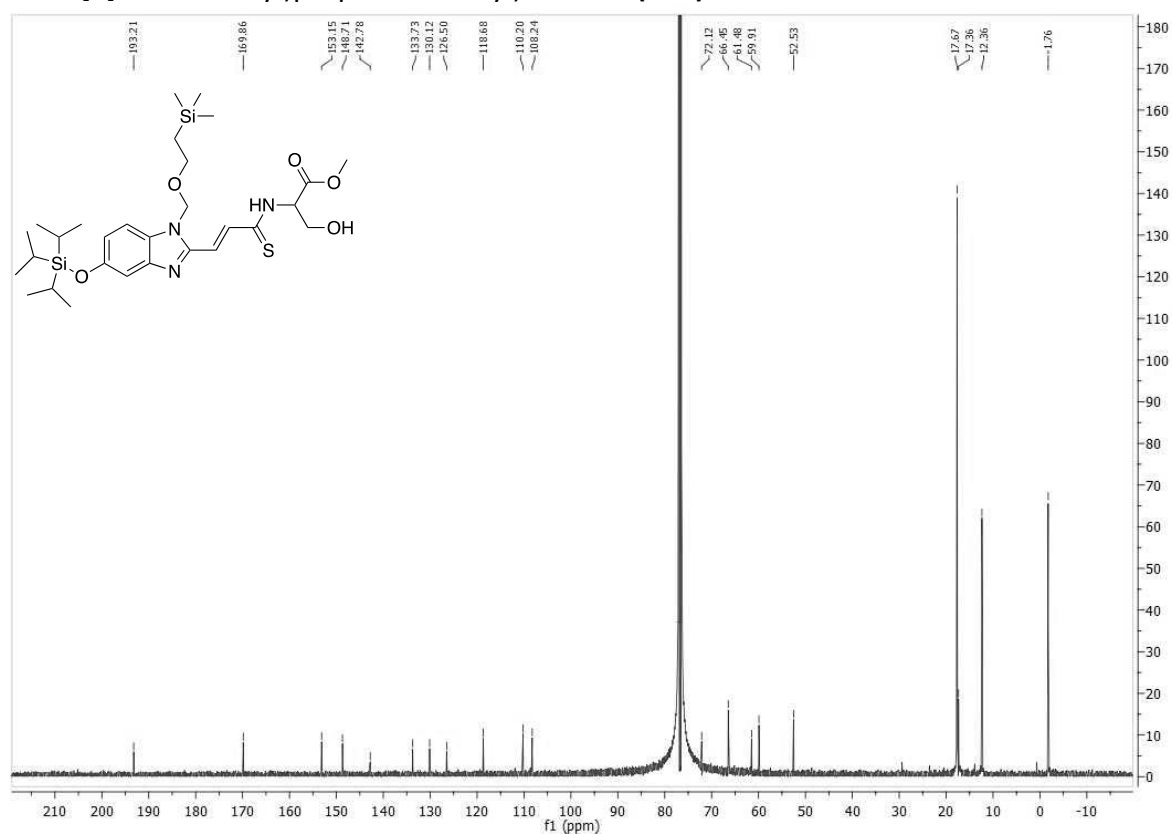
**<sup>13</sup>C-NMR:** methyl (*E*)-(3-(6-((triisopropylsilyl)oxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)prop-2-enethioyl)serinate (**26a**)



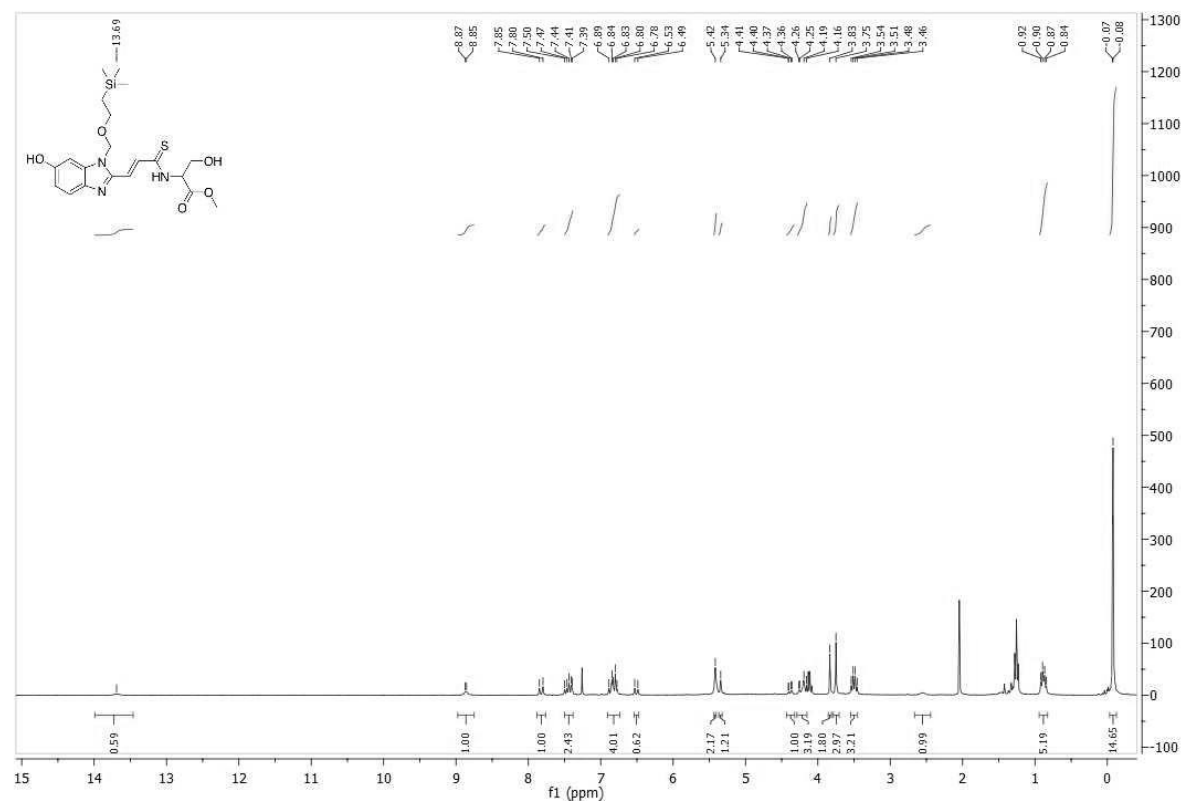
**<sup>1</sup>H – NMR:** methyl(*E*)-(3-(5-((triisopropylsilyl)oxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)prop-2-enethioyl)serinate (**26b**)



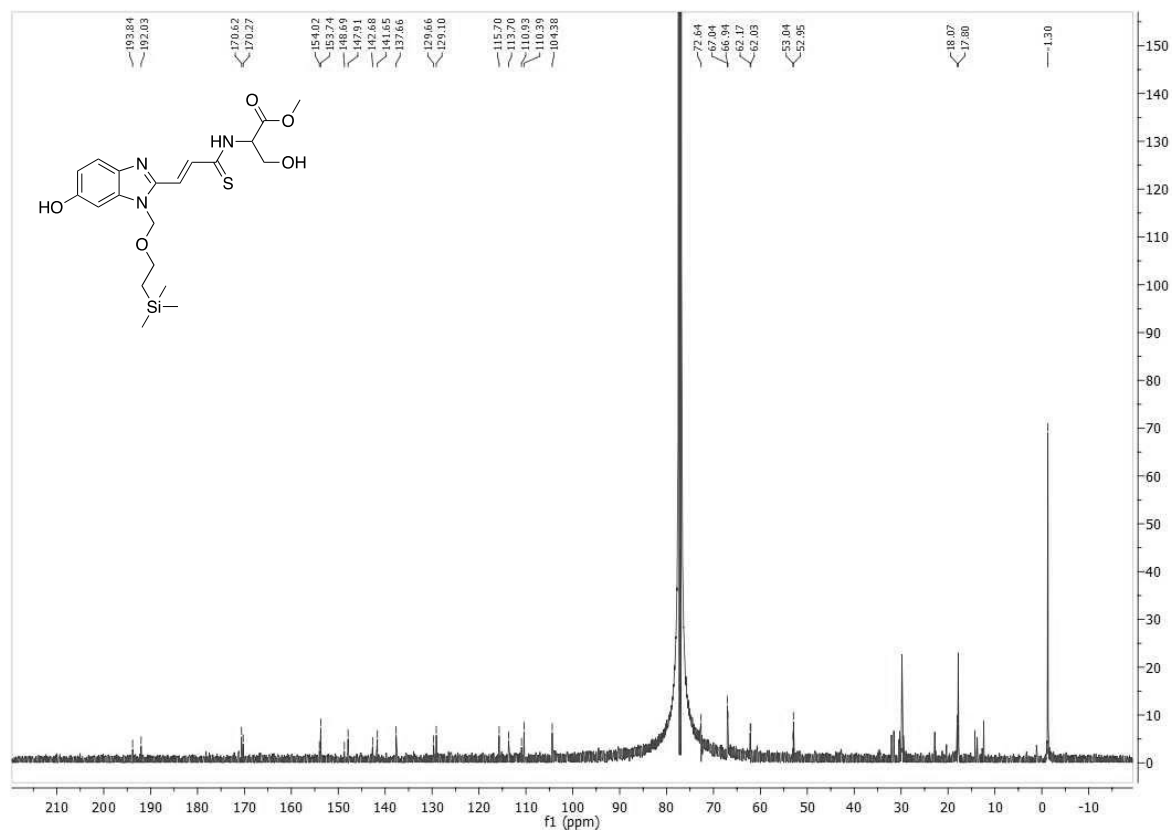
**<sup>13</sup>C-NMR:** methyl(*E*)-(3-(5-((triisopropylsilyl)oxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)prop-2-enethioyl)serinate (**26b**)



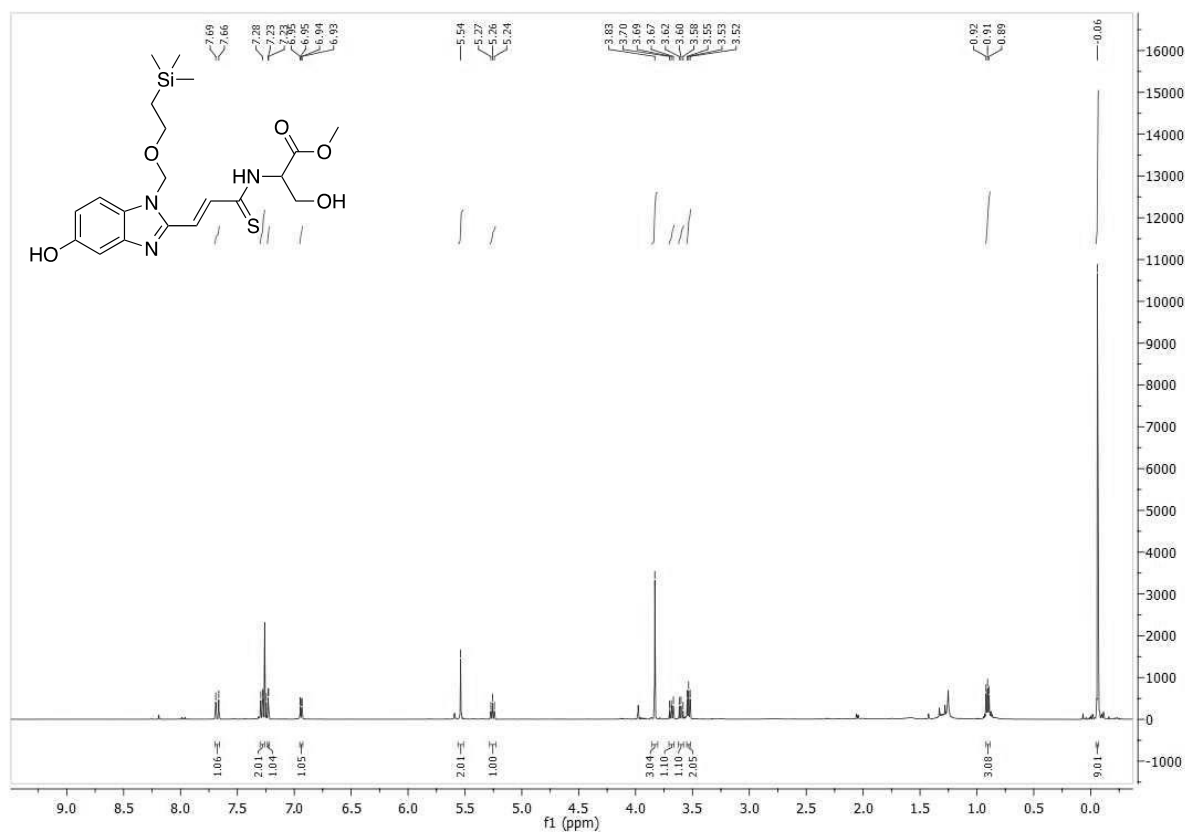
**<sup>1</sup>H – NMR:** methyl (*E*)-(3-(6-hydroxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)prop-2-enethioyl)serinate (**27a**)



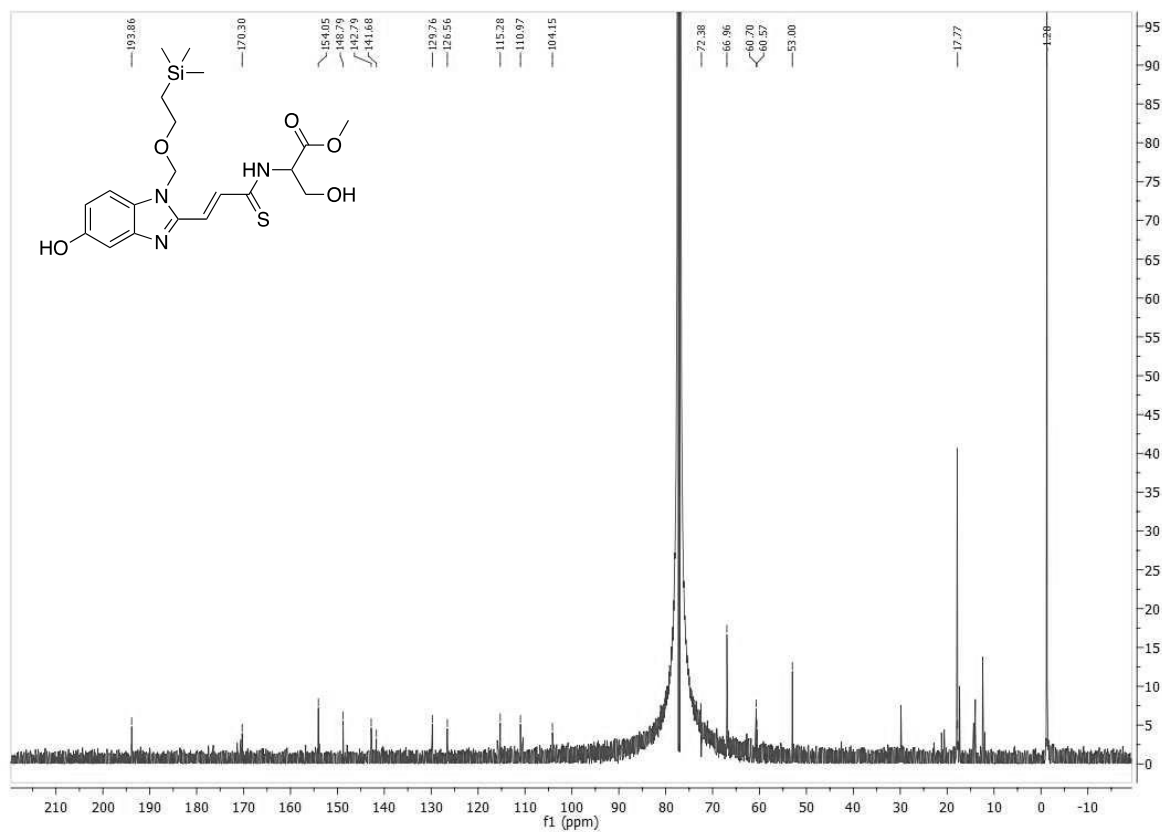
**<sup>13</sup>C-NMR:** methyl (*E*)-(3-(6-hydroxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)prop-2-enethioyl)serinate (**27a**)



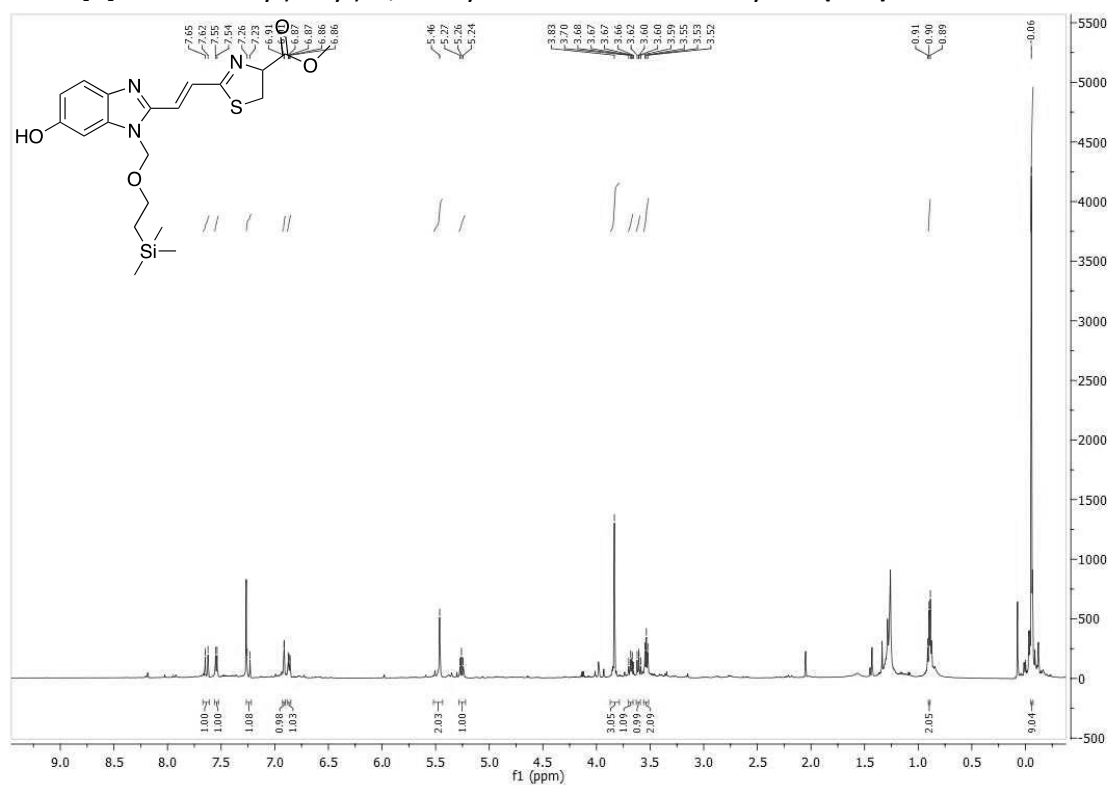
**<sup>1</sup>H – NMR:** methyl (*E*)-(3-(5-hydroxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)prop-2-enethioyl)serinate (**27b**)



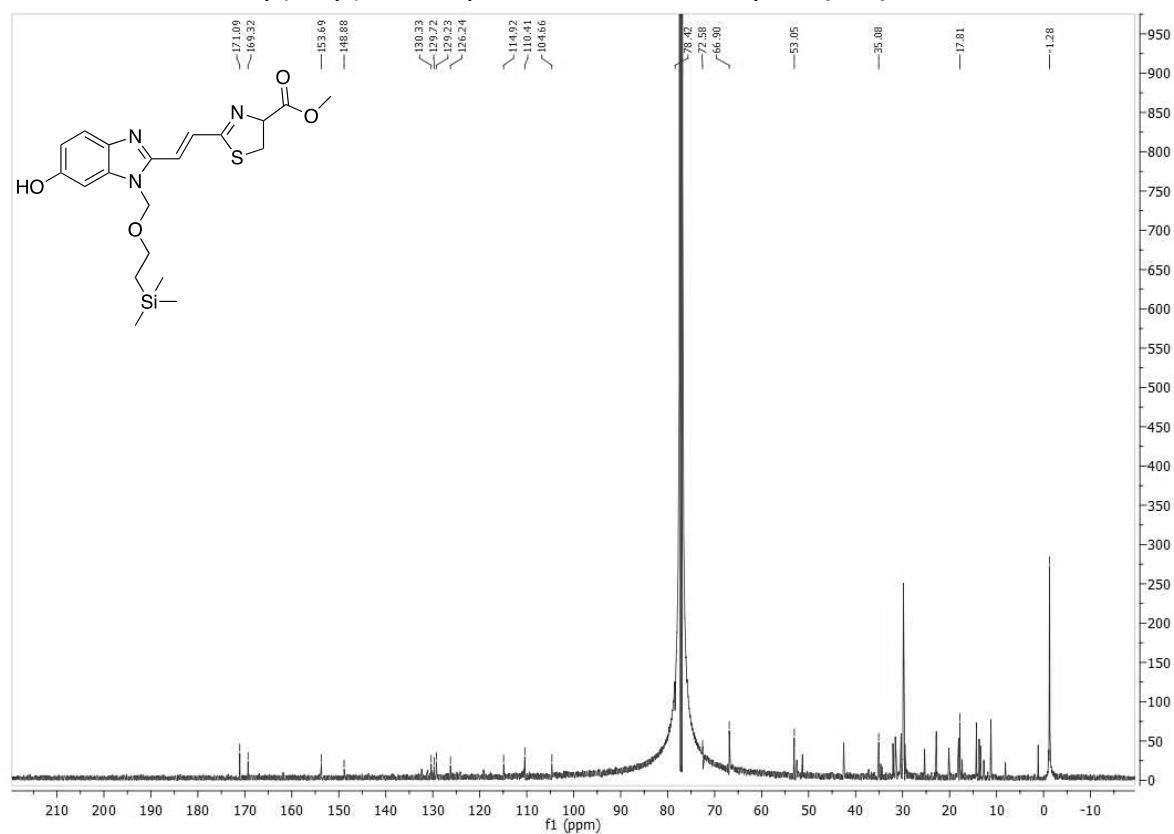
**<sup>13</sup>C-NMR:** methyl (*E*)-(3-(5-hydroxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)prop-2-enethioyl)serinate (**27b**)



**<sup>1</sup>H – NMR:** methyl (*E*)-2-(2-(6-hydroxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylate (**28a**)

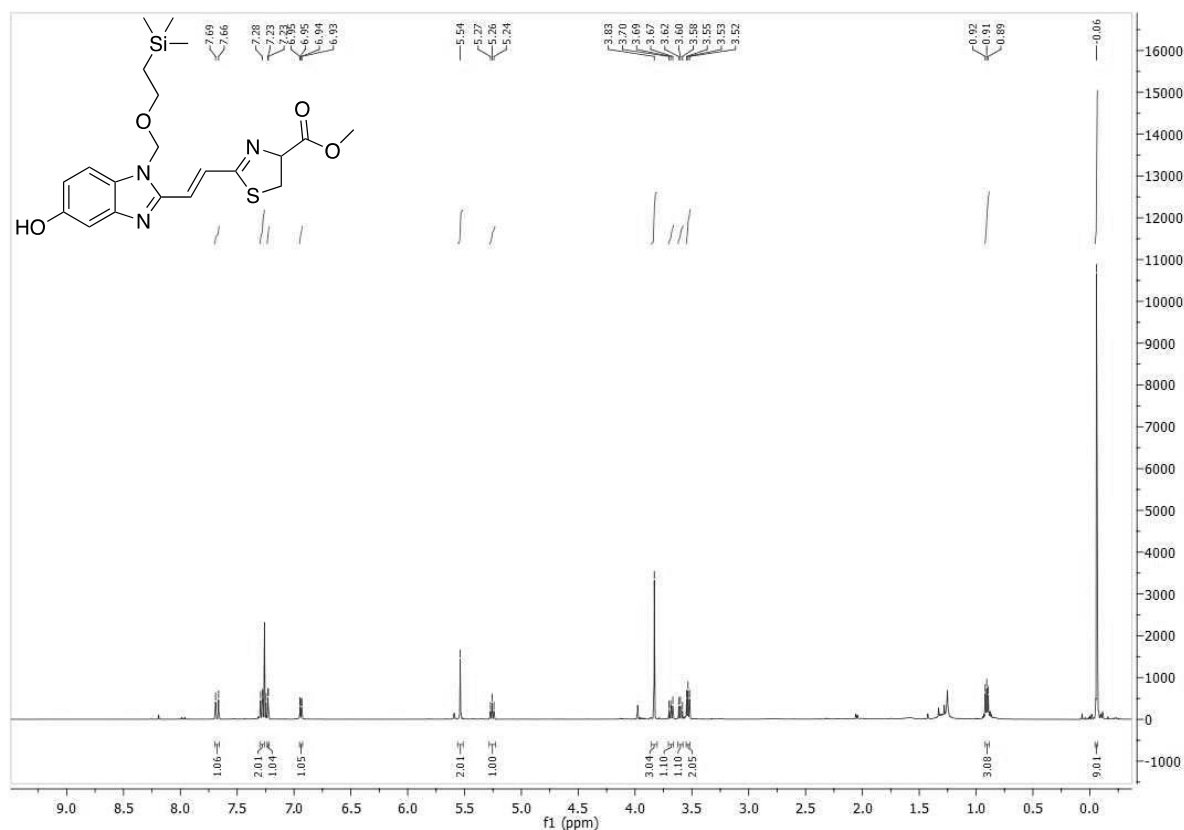


**<sup>13</sup>C-NMR:** methyl (*E*)-2-(2-(6-hydroxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylate (**28a**)

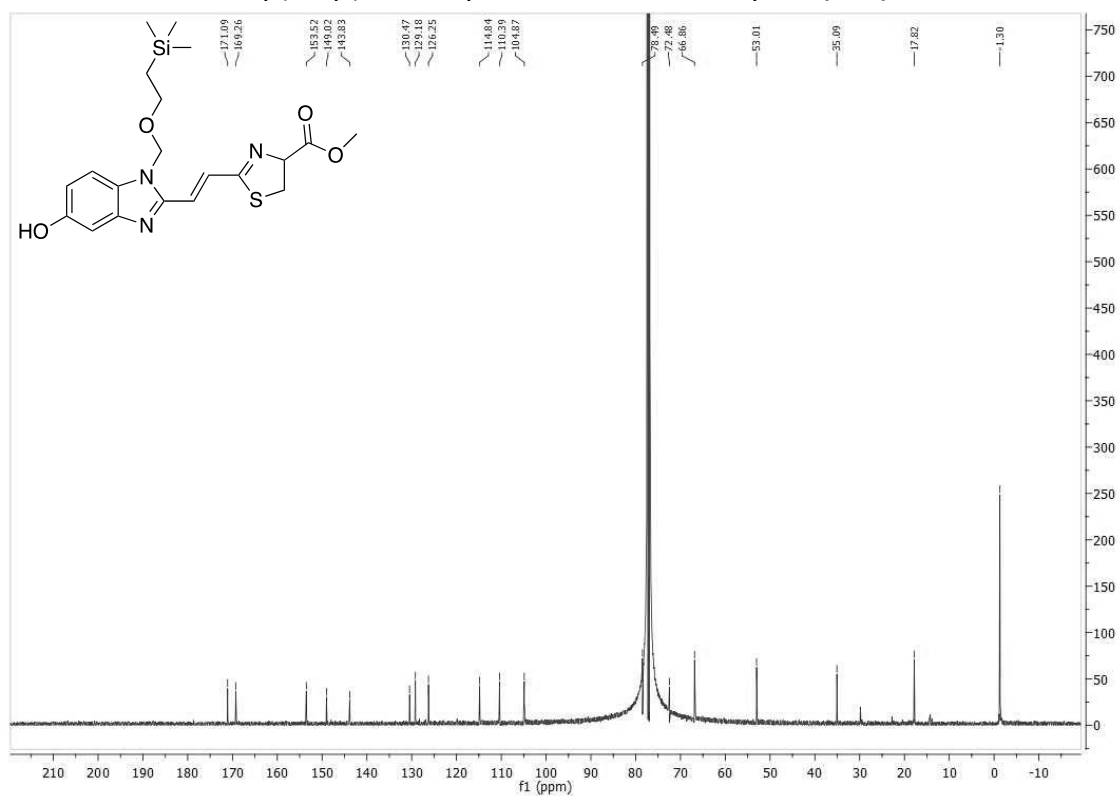




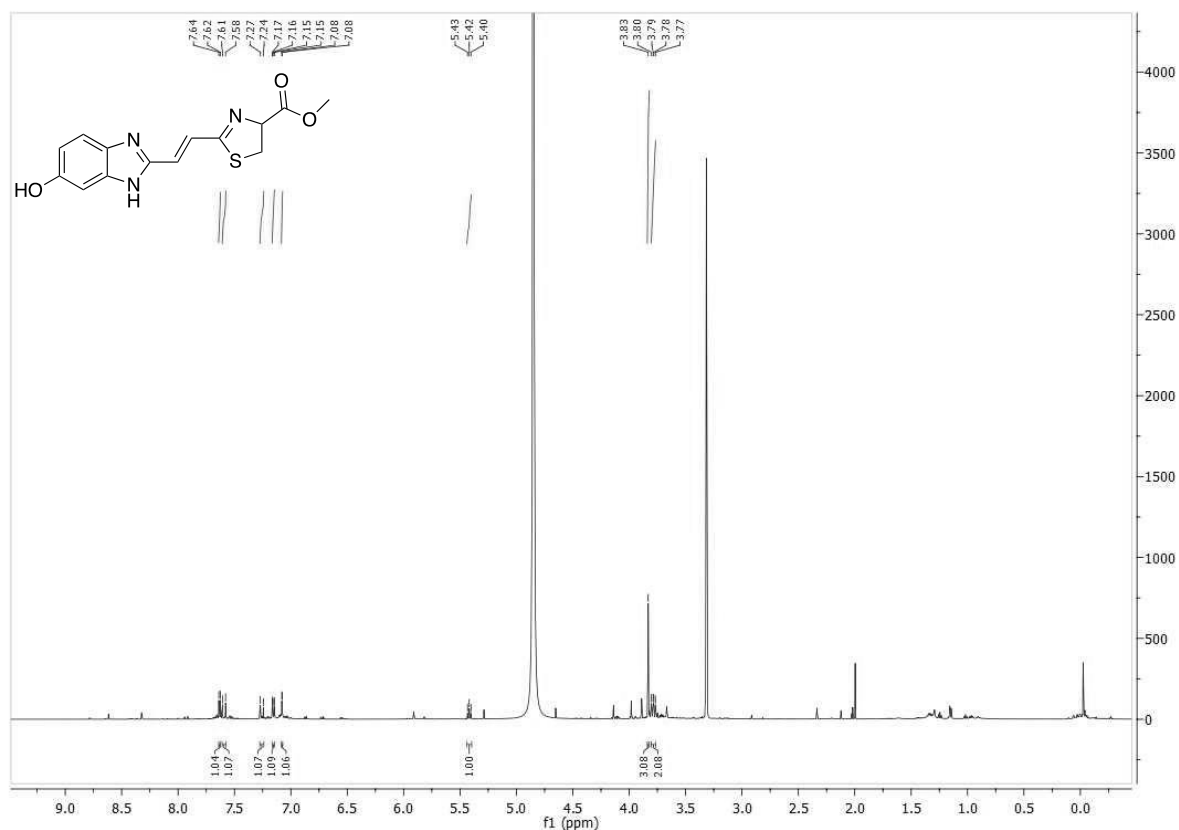
**<sup>1</sup>H – NMR:** methyl (*E*)-2-(2-(5-hydroxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylate (**28b**)



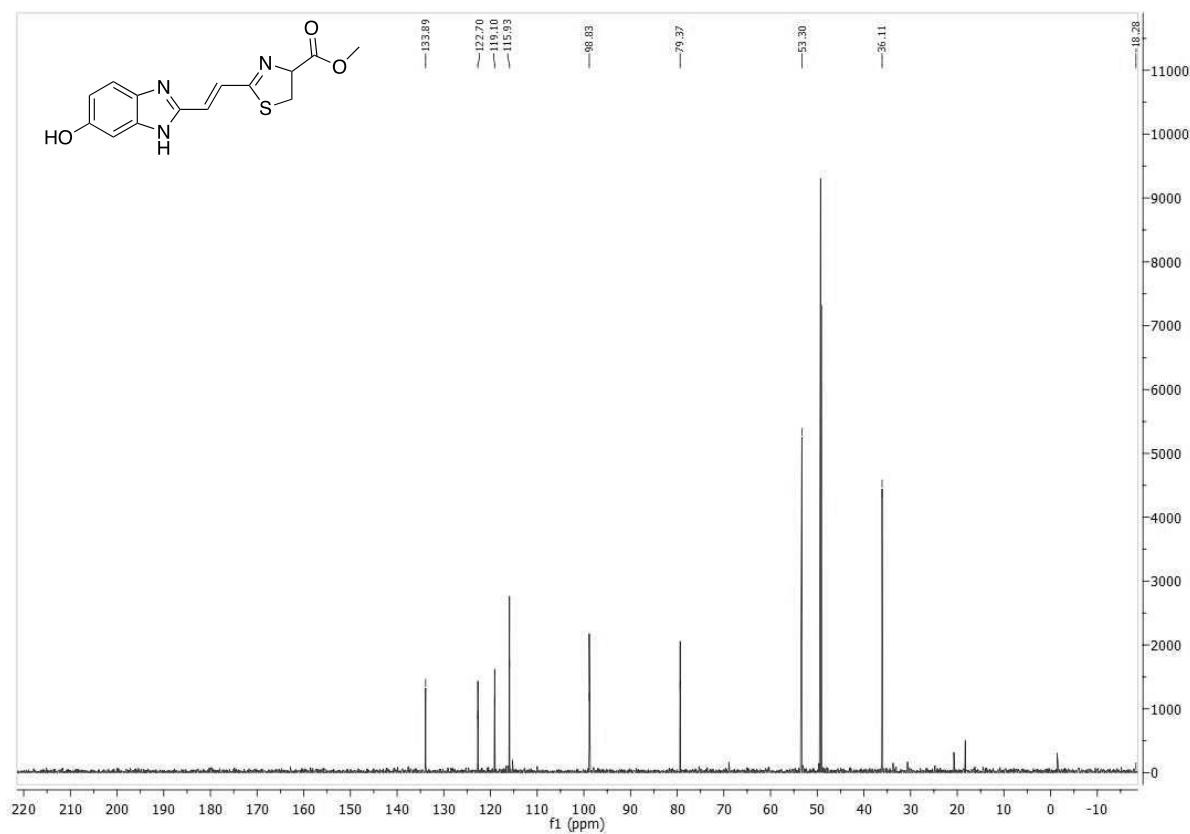
**<sup>13</sup>C-NMR:** methyl (*E*)-2-(2-(5-hydroxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylate (**28b**)



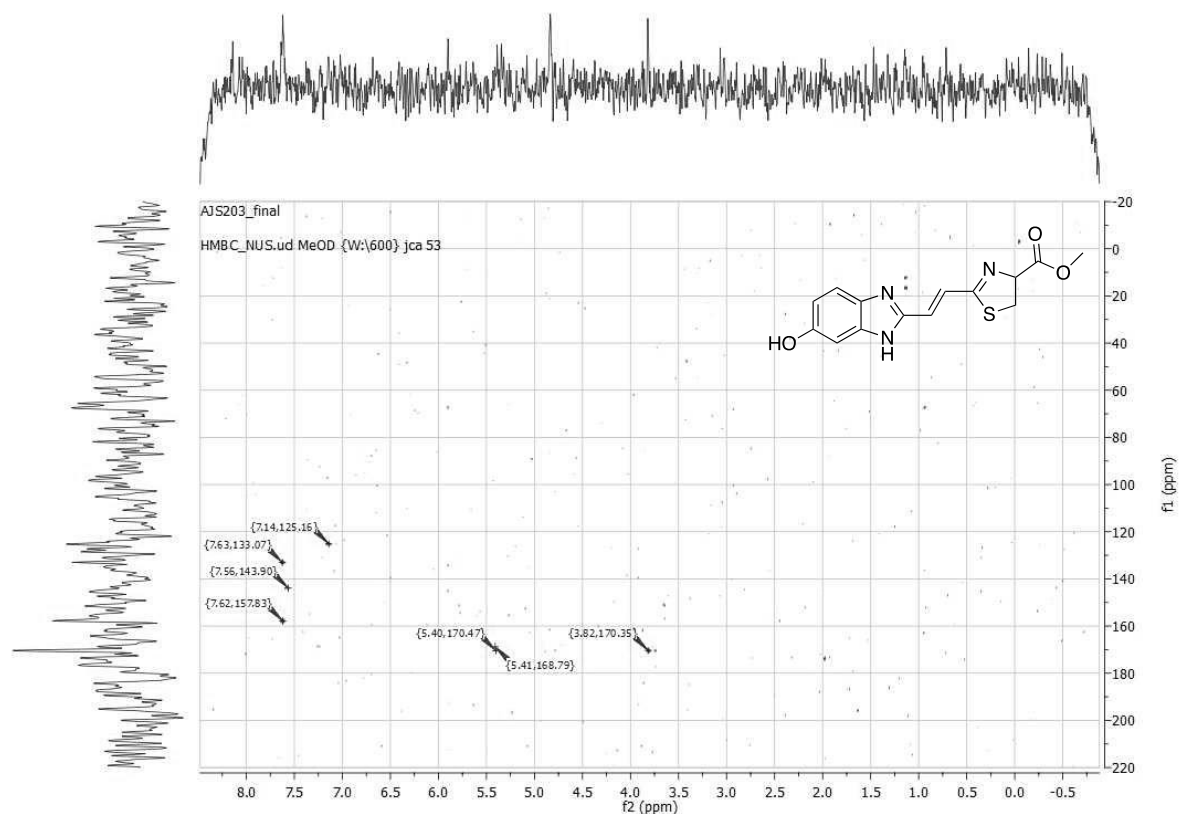
**<sup>1</sup>H – NMR:** methyl (*E*)-2-(2-(6-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylate



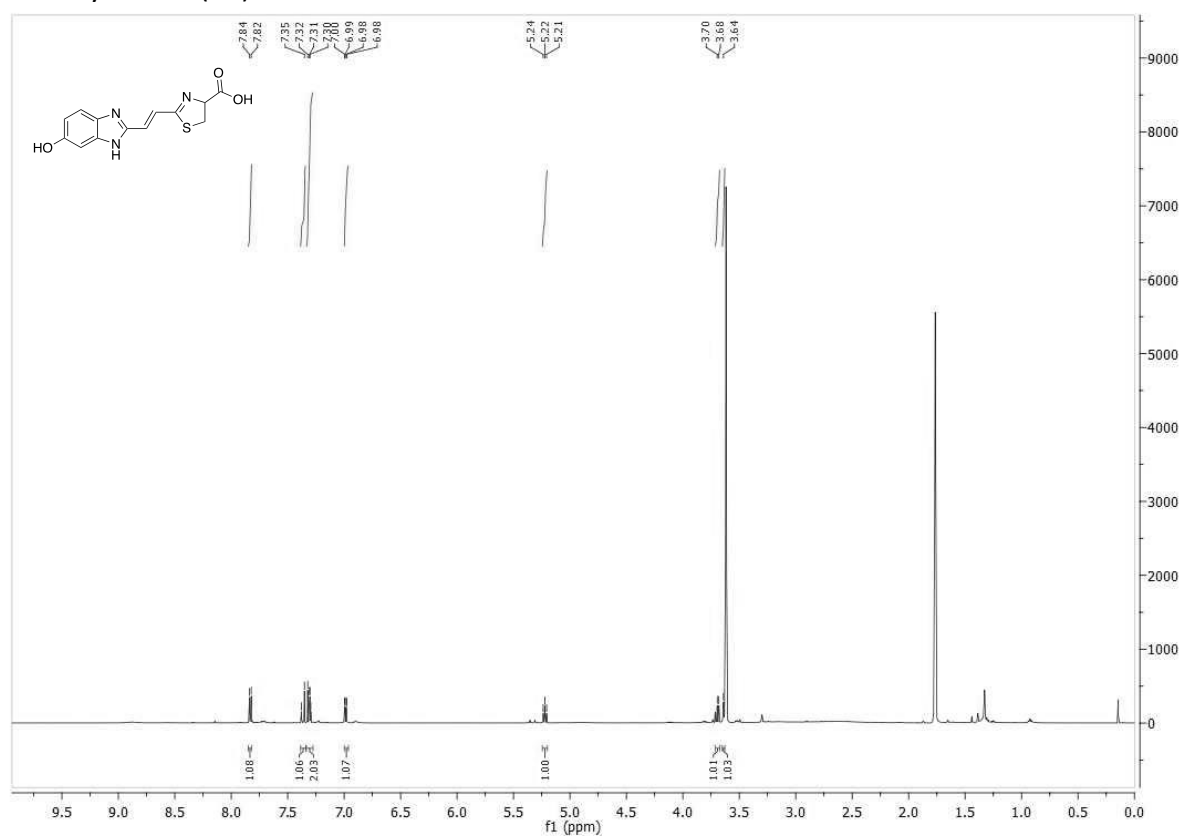
**<sup>13</sup>C-NMR:** methyl (*E*)-2-(2-(6-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylate



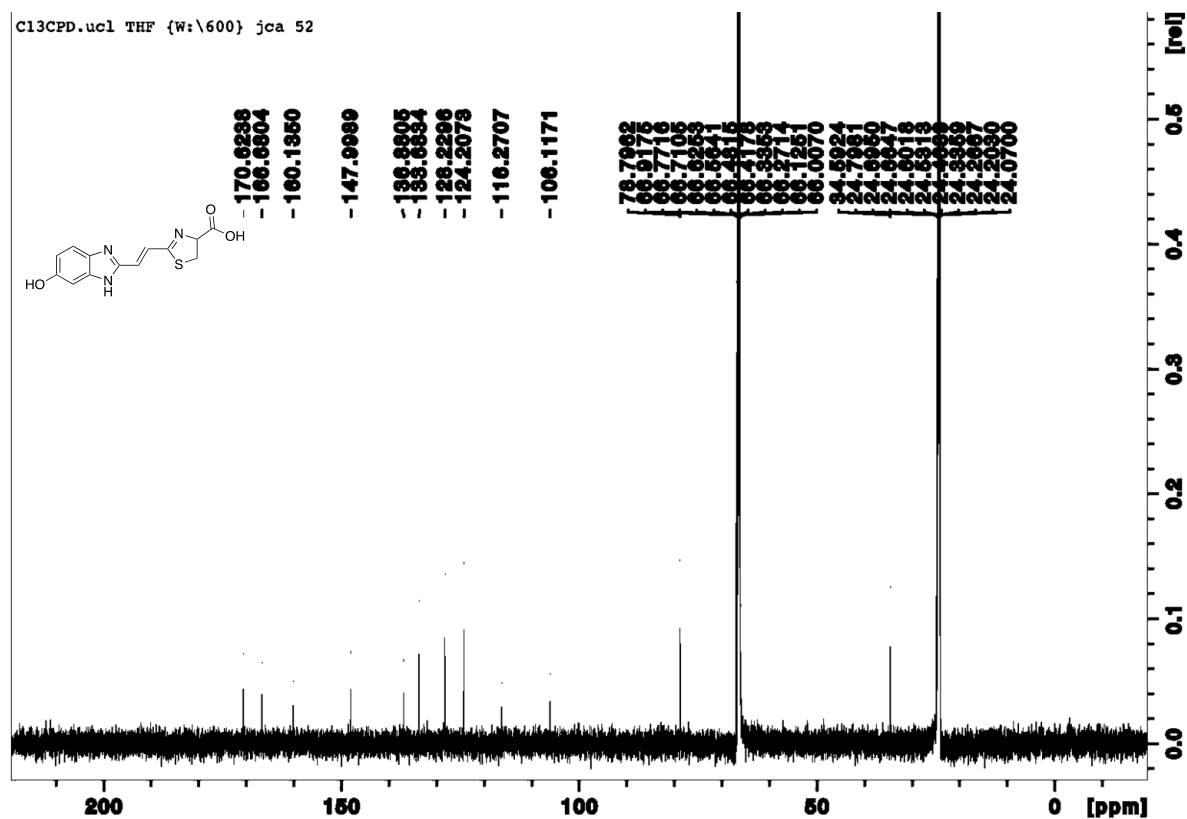
**HMBC spectrum:** methyl (*E*)-2-(2-(6-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylate



**<sup>1</sup>H – NMR:** (*E*)-2-(2-(6-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylic acid (**13**)



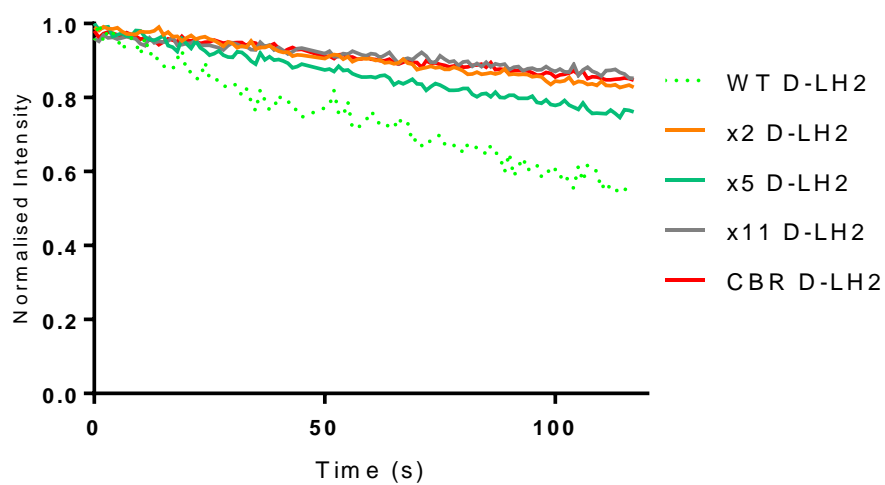
**<sup>13</sup>C-NMR:** (*E*)-2-(2-(6-hydroxy-1*H*-benzo[*d*]imidazol-2-yl)vinyl)-4,5-dihydrothiazole-4-carboxylic acid (**13**)



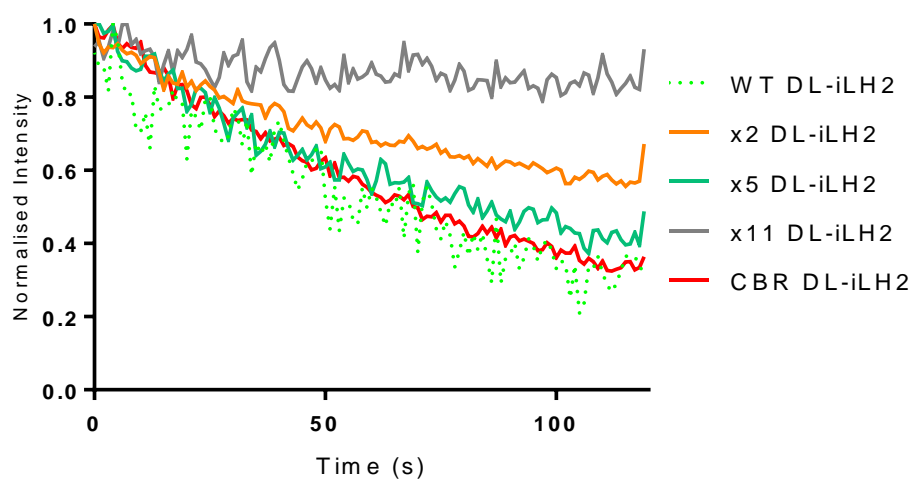
# Emission kinetics of enzymes with different compounds acquired in the PIO

SI Figure:

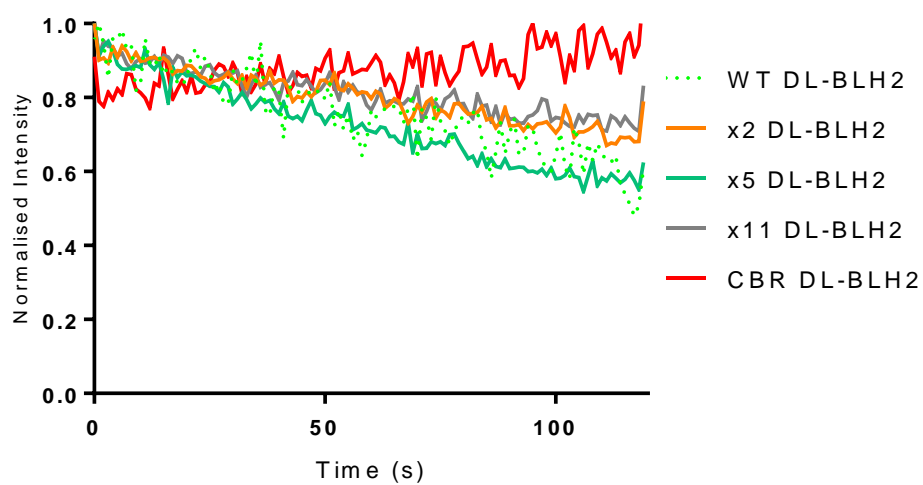
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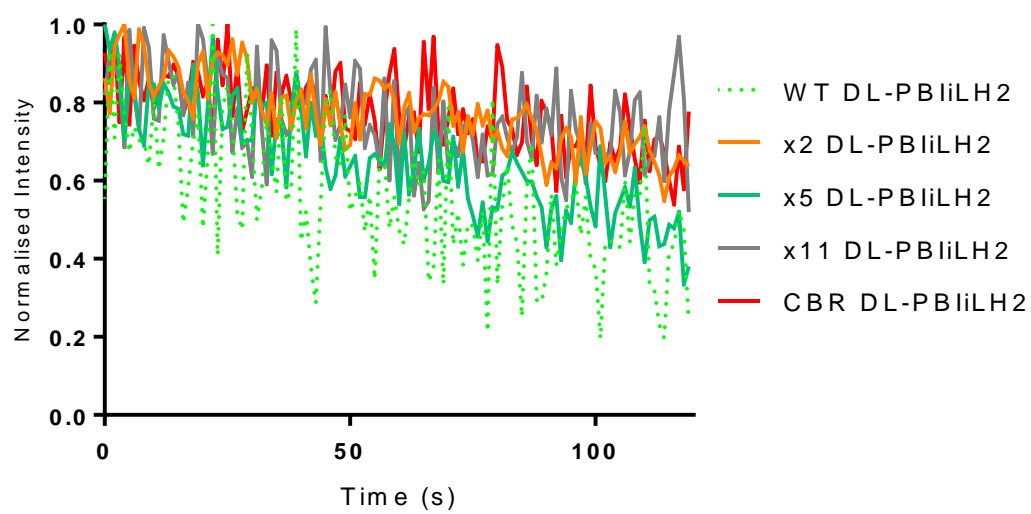
B



C



D



E

