Supporting information

Semisynthetic bioluminescent sensor proteins for direct detection of antibodies and small molecules in solution

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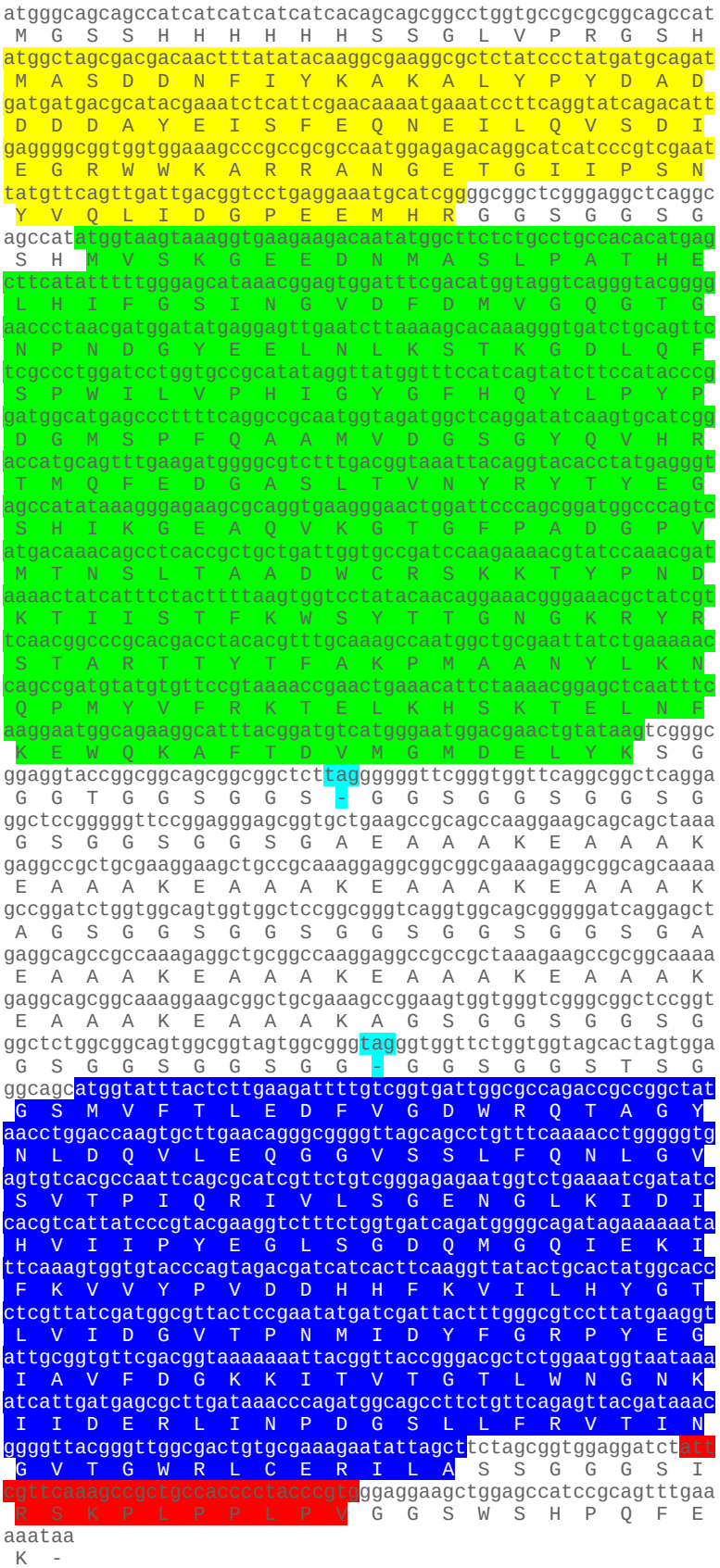


Figure S1: DNA and amino acid sequence of TAG-LUMABS. Areas of interest are indicated as follows: SH3 domain (yellow), mNeonGreen (green), TAG codons (cyan), NanoLuc (blue), proline-rich peptide (red).

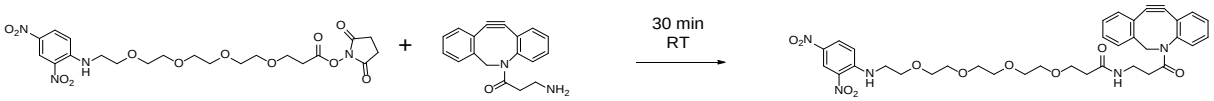
Supplementary methods

Synthesis of DBCO-functionalized small molecules

General

All used solvents were obtained from Biosolve BV and used without further purification. Water was purified using a Millipore purification train. LC-MS was performed on a C4, Jupiter SuC4300A, 150x2.00 mm column using H_2O with 0.1 % formic acid and acetonitrile with 0.1% formic acid using a gradient of 5% to 100% acetonitrile in 10 min and a flow rate of 0.2 mL/min. Preparative HPLC was performed on a Gemini S4 110A 150x21.20 mm column using H_2O with 0.1% F.A. and acetonitrile with 0.1% F.A, with gradients as indicated per synthesis. NMR spectroscopy data was recorded with a Bruker Advance-III 400 MHz using a BBFO probe from Bruker (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts are reported in parts per million (ppm) referenced to an internal standard of residual [D]chloroform (δ = 7.26 ppm for ^{1}H and δ = 77 ppm for ^{13}C, relative to tetramethylsilane as an internal standard).

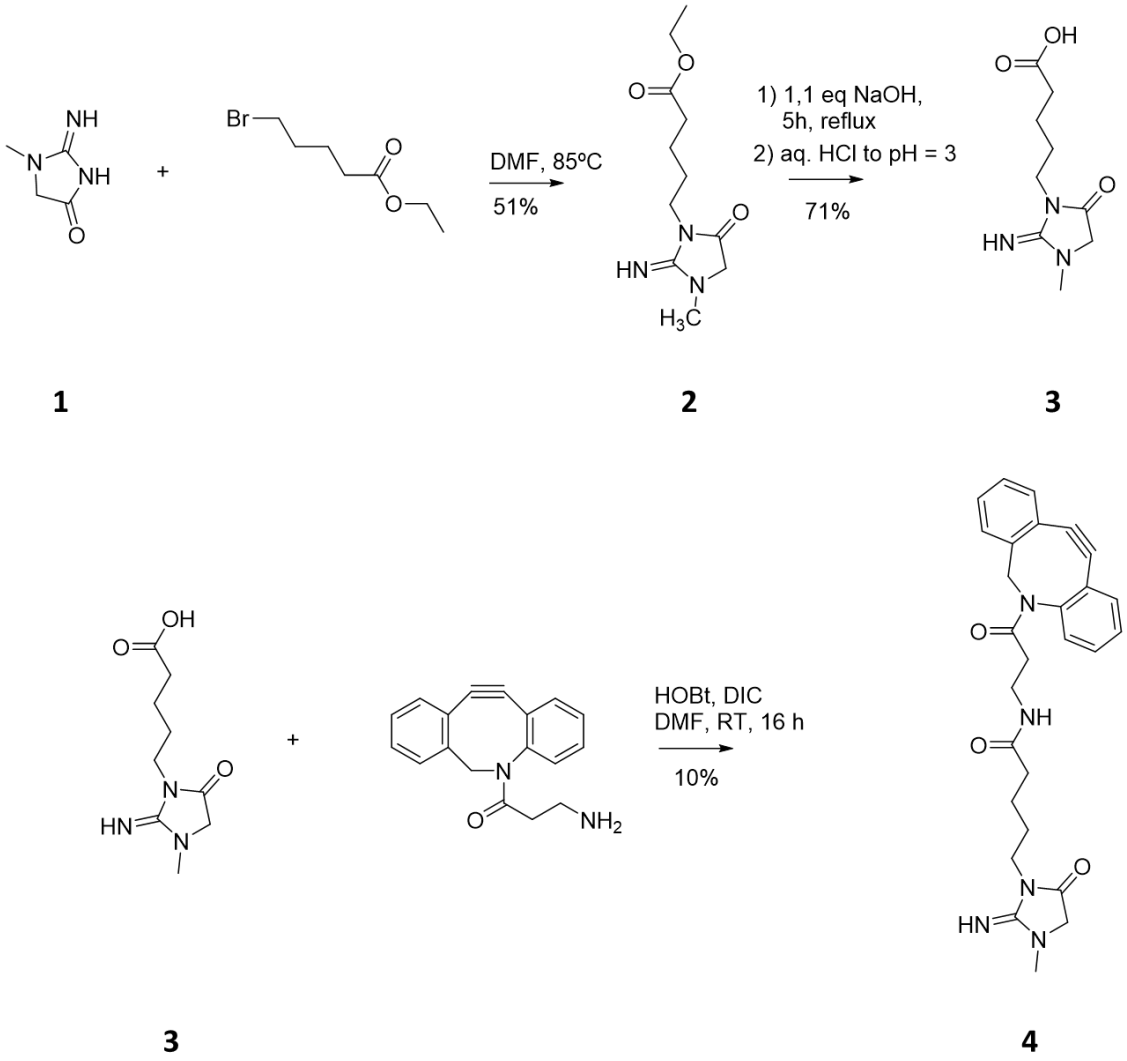
DNP-DBCO



Scheme S1: *Synthesis of DBCO-PEG-DNP.*

2 mg (7 μmol) DBCO-NH2 (Sigma-Aldrich, 761540-10MG) was coupled to DNP-dPEG4-NHS ester (Quanta Biodesign, 10347, 1.5 equivalents) in a 30 minute reaction in PBS (pH = 7.2) in a total volume of 2.25 mL at room temperature. This product was purified using preparative HP-LC (60-80% ACN/H_2O gradient). Product purity was confirmed using LC-MS (MW_{calc} = 689.72, MW_{obs} = 690.42).

CR-DBCO



Scheme S2: *Synthesis of DBCO-creatinine*

DBCO-NH_2 was coupled to creatinine in a three-step synthesis based on the protocol reported by Binkert *et al.* (see Scheme S2).[1] 8 mmol of creatinine (Sigma-Aldrich; C4255) was dissolved in 5 mL dimethylformamide (DMF) in a round-bottomed flask fitted with a magnetic stirring bar and heated with stirring (in an oil bath) until completely dissolved. 1.25 equivalent of 5-ethyl-bromovalerate (Sigma-Aldrich; cat no. 14660-52-7) was added to the reaction mixture and left to react for 16 hours. The product (**2)** was obtained via precipitation in ethylacetate and isolated by filtration. The reaction mixture was further purified by silica gel (40-63 μm) column purification (1:9 MeOH:$CHCl_3$). The isolated creatinine ester (**2**) was characterized by LC-MS (Figure S3) and NMR spectroscopy (Figure S5-9). To obtain the

creatinine-acid (**3**), the creatinine ester (**2**) produced in the previous was dissolved in 3 mL of H_2O, treated with 1.1 equivalent NaOH, and the reaction heated overnight under reflux. After cooling to room temperature the pH of the reaction mixture was adjusted to pH = 3 through the addition of 0.2 M aq. HCl. The creatinine acid was treated with 1 equivalent of diisopropylcarbodiimide (DIC), to activate the carboxylic acid, and left to stir at room temperature for 20 minutes. 1 equivalent hydroxybenzotriazole (HOBt) was then added, and the reaction stirred for an additional 20 minutes. Finally, 0.2 equivalent DBCO-amine was added and the reaction monitored with LC/MS (Figure S4). The final product, DBCO-creatinine (**4**), was purified using preparative LC-MS (23-55% ACN/H_2O gradient) and characterized by LC-MS: MW_{calc} = 471.56, MW_{obs} = 472.17 (Figure S4).

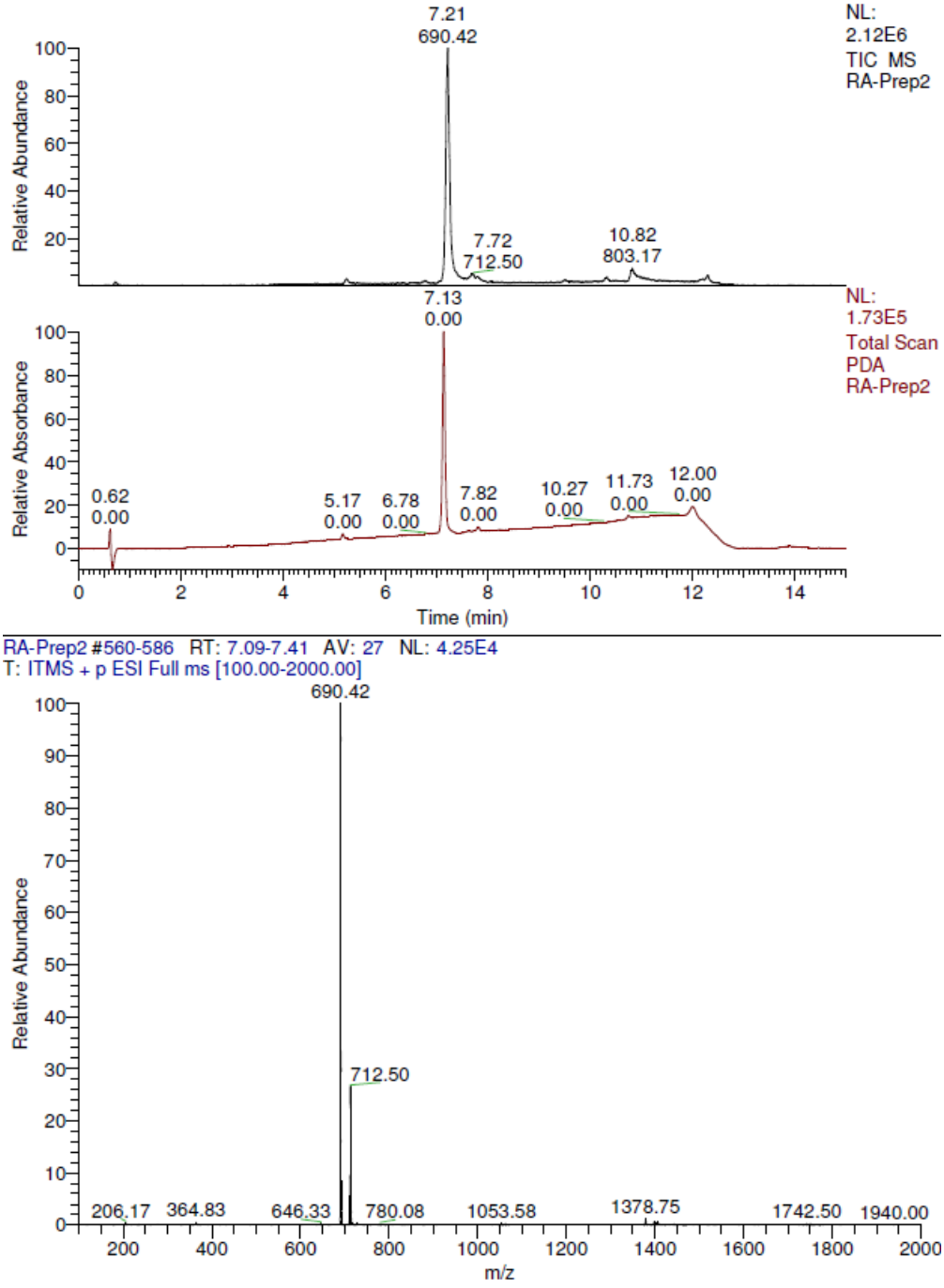


Figure S2: LC/MS of DNP-DBCO. Top: total ion count. Middle: UV-trace. Bottom: Mass spectrum. Expected mass: $[M+H]^+$ = 690.42 Da.

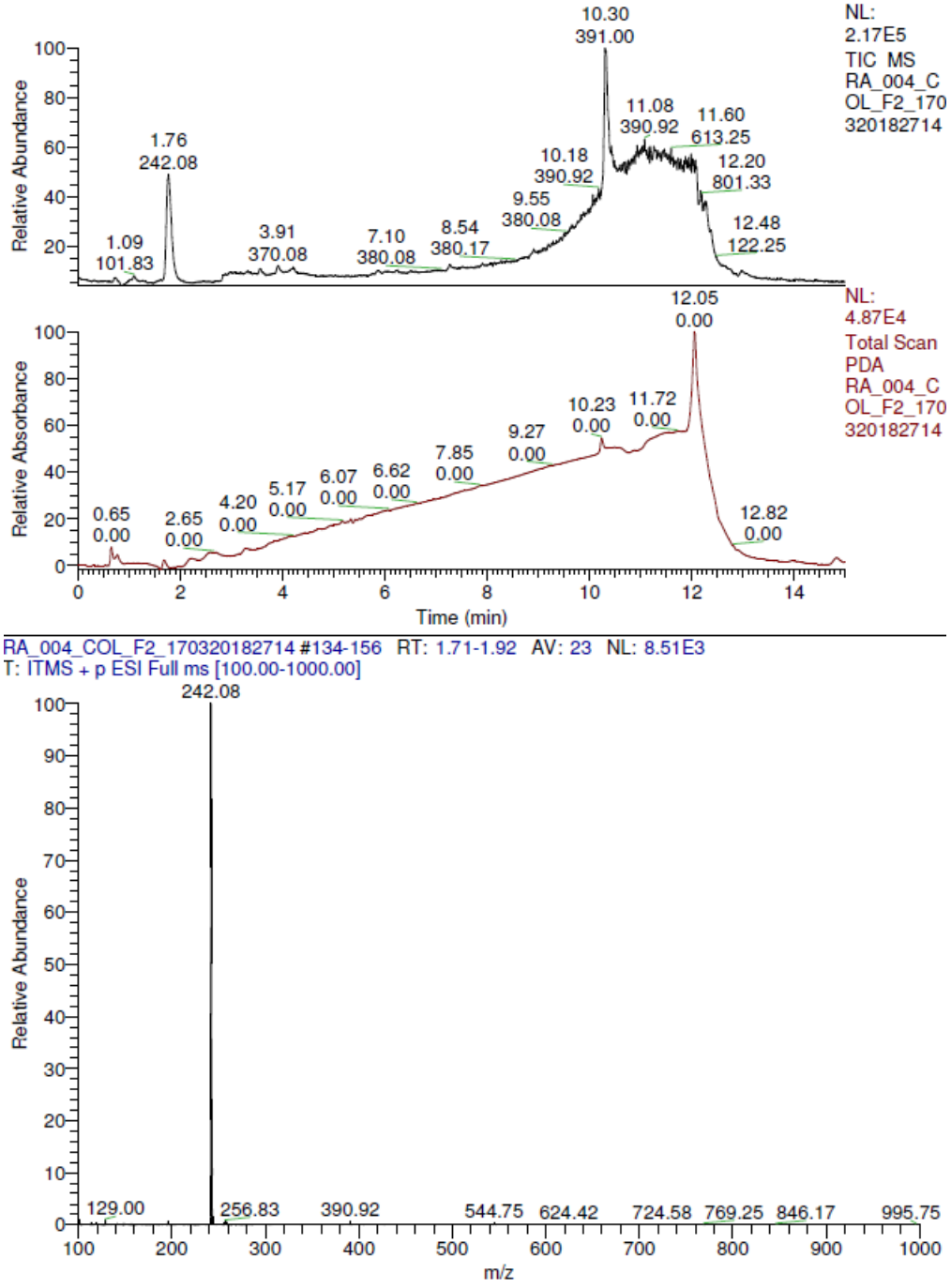


Figure S3: LC/MS of creatinine ester **2**. Top: total ion count. Middle: UV-trace. Bottom: Mass spectrum. Expected mass: $[M+H]^+$ = 242.08 Da.

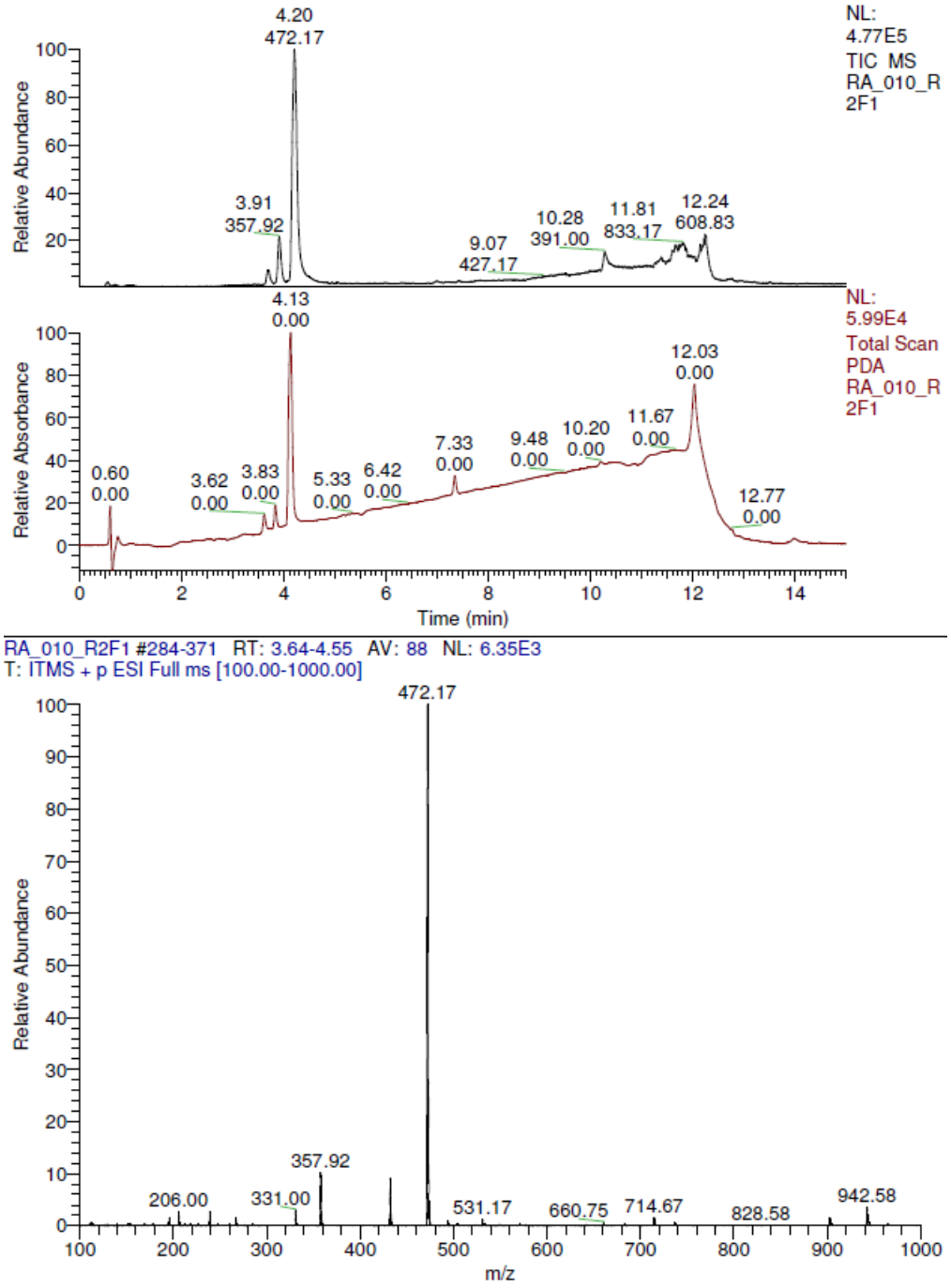


Figure S4: LC/MS of DBCO-creatinine **4**. Top: total ion count. Middle: UV-trace. Bottom: Mass spectrum. Expected mass: $[M+H]^+$ = 472.17 Da. Compound **4** was obtained with a purity of 85.7%.

NMR Spectra of creatinine ester (Compound 2)

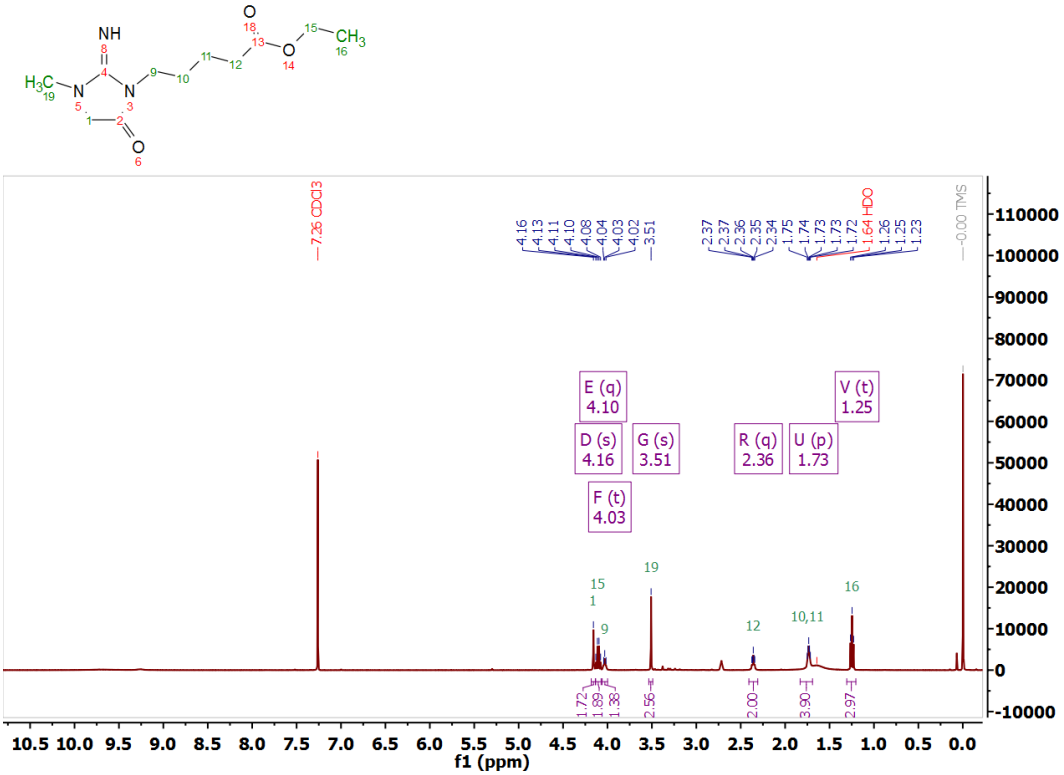


Figure S5: [1]H NMR of Compound **2 (**400 MHz, Chloroform-*d*) δ 4.16 (s, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 4.03 (t, *J* = 5.2 Hz, 1H), 3.51 (s, 3H), 2.36 (q, *J* = 5.0, 3.6 Hz, 2H), 1.73 (p, *J* = 3.6 Hz, 4H), 1.25 (t, *J* = 7.1 Hz, 3H).

[13]C NMR

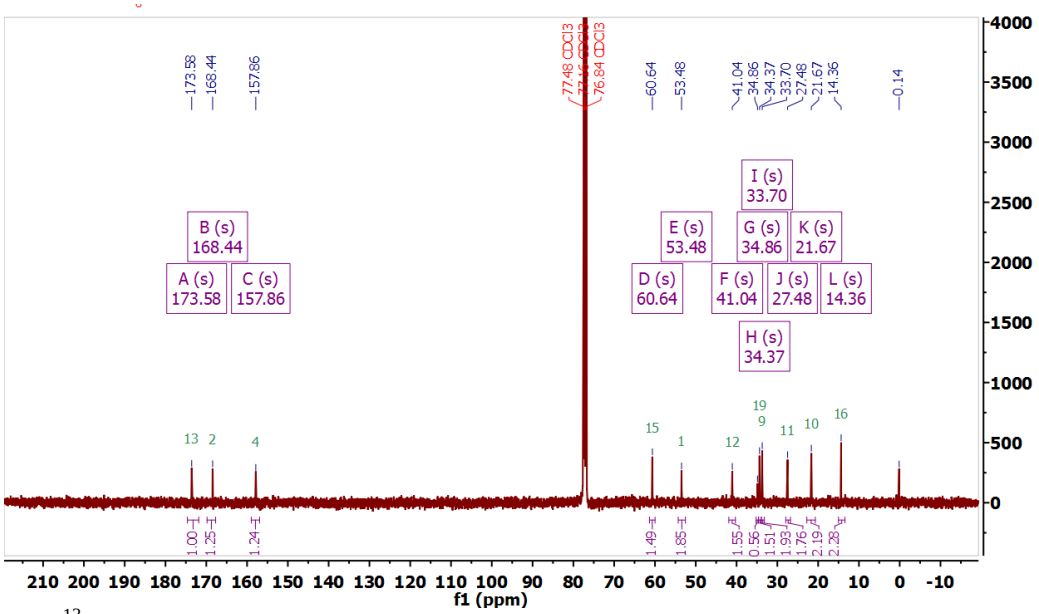


Figure S6: [13]C NMR of Compound **2** (101 MHz, Chloroform-*d*) δ 173.58, 168.44, 157.86, 60.64, 53.48, 41.04, 34.86, 34.37, 33.70, 27.48, 21.67, 14.36.

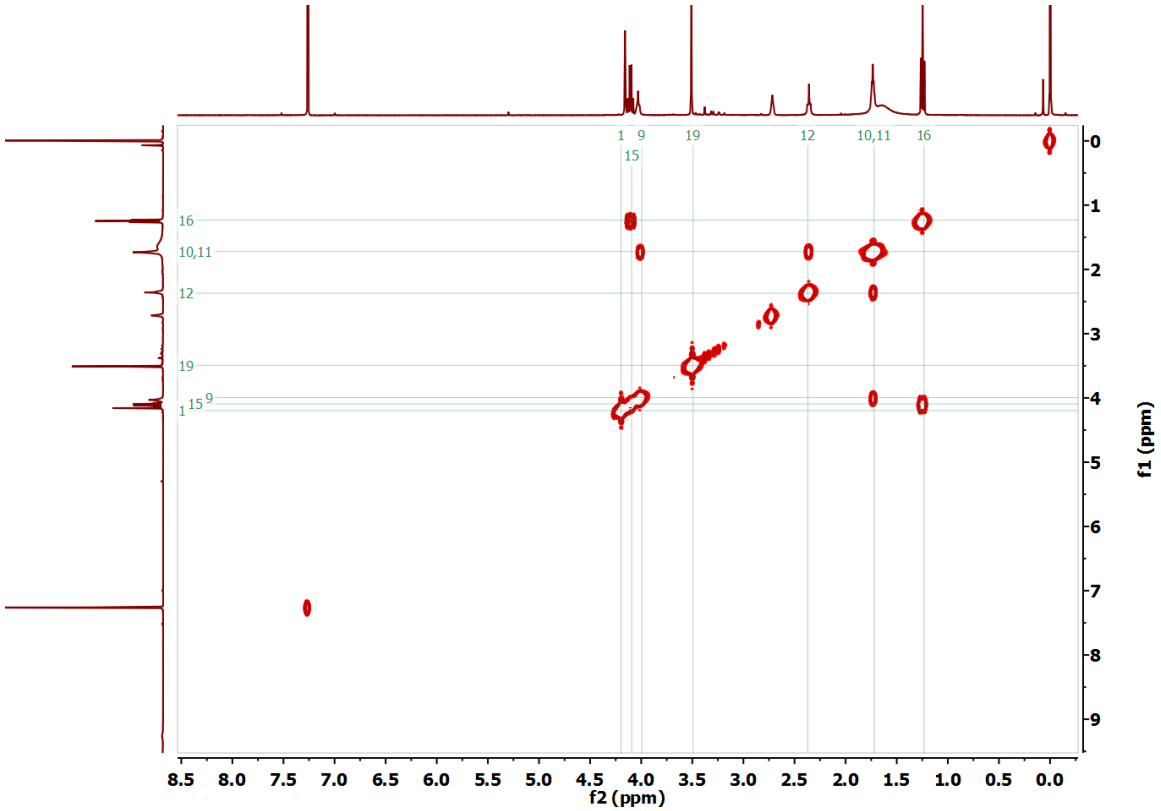


Figure S7: ^{1}H COSY of Compound **2**.

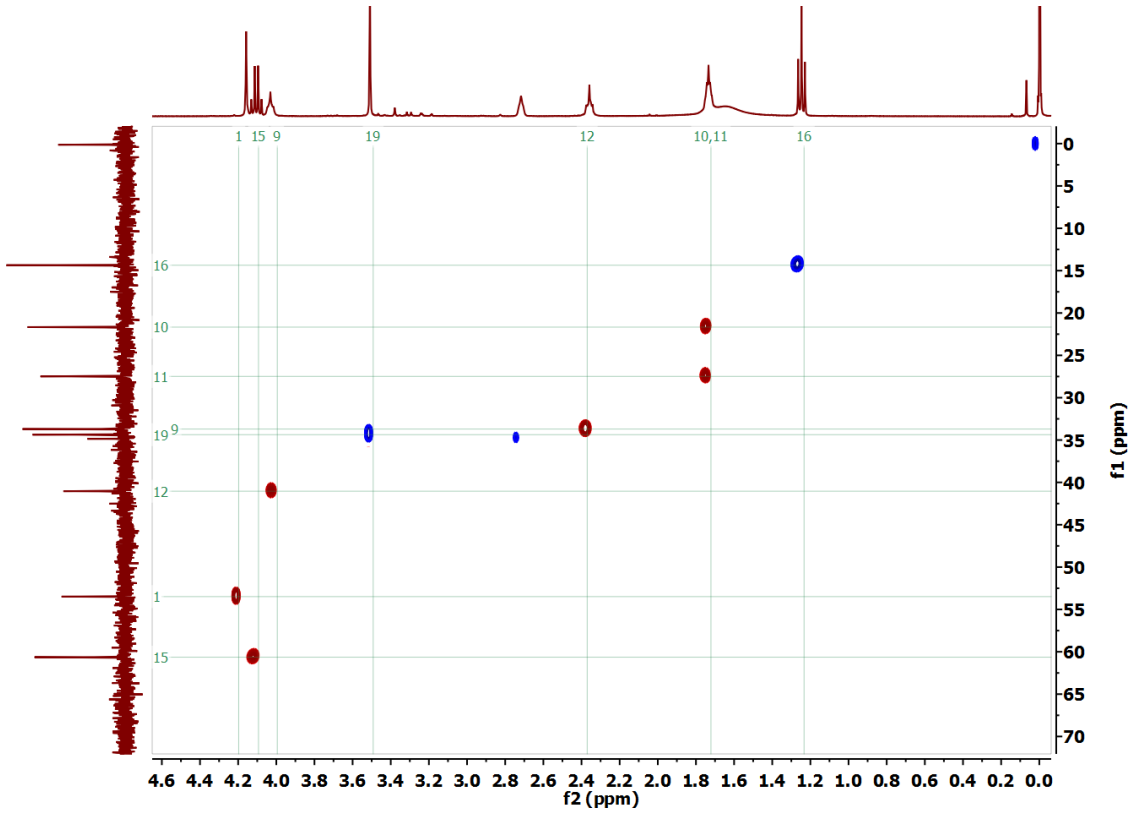


Figure S8: ^{1}H-^{13}C HSQC of Compound **2**.

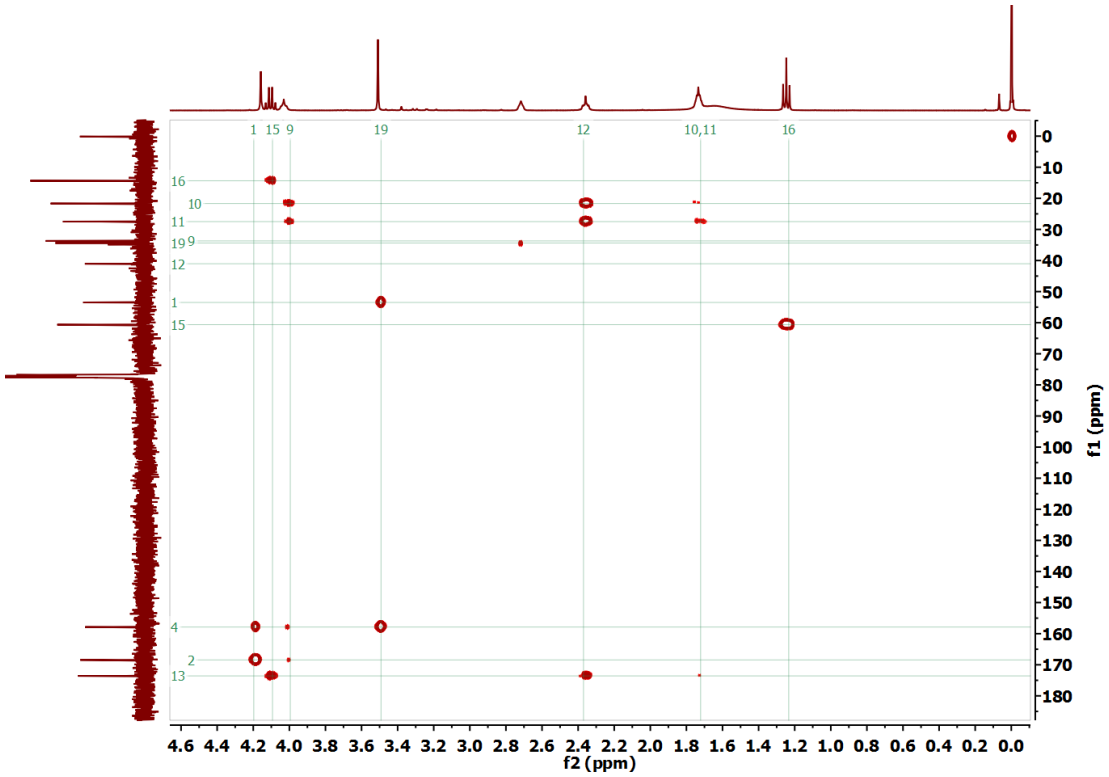


Figure S9: ^{1}H-^{13}C HMBC of compound **2**.

Table S1. Assignment of Compound **2** protons and their correlations in ^{1}H-COSY and ^{1}H-^{13}C HMBC.

Assignment	Shift	Range	H's	Integral	Class	J's	^{1}H-COSY	^{1}H-^{13}C HMBC
16	**1.25**	1.28 .. 1.22	3	3.00	**t**	7.12, 7.12	H16-H15	H16-C15 C16-H15
10, 11	**1.73**	1.83 .. 1.69	4	3.90	**p**	3.62, 3.62, 3.90, 3.90	H10/H11-H12 H10/H11-H9	H11-C10 H11-C11 C10-H9 C10-H12 C10-H10/H11 C11-H9 C11-H10/H11 C11-H12
12	**2.36**	2.41 .. 2.31	2	2.00	**q**	5.02, 5.02, 3.61	H12-H10/H11	H12-C10 H12-C11 H12-C13
19	**3.51**	3.53 .. 3.49	3	2.56	**s**			H19-C1 H19-C4
9	**4.03**	4.06 .. 4.00	1	1.38	**t**	5.20, 5.20	H9-H10/H11	H9-C2 H9-C4 H9-C10 H9-C11
15	**4.10**	4.14 .. 4.07	2	1.89	**q**	7.18, 7.18, 7.17	H15-H16	H15-C13 H15-C16 C15-H16
1	**4.16**	4.18 .. 4.14	2	1.82	**s**			H1-C2 H1-C4 C1-H19

The assignment of the proton and carbon peaks of Compound **2** was performed using ^{1}H NMR, ^{13}C NMR, ^{1}H COSY and ^{1}H-^{13}C HSQC. The peak appearing at 2.75 ppm in ^{1}H-NMR and at 34.86 ppm in the ^{13}C NMR presumably corresponds to an impurity that could not be assigned. The regioselectivity of the reaction was confirmed using ^{1}H-^{13}C HMBC.

References

1 A. Benkert, F. Scheller, W. Schössler, C. Hentschel, B. Micheel, O. Behrsing, G. Scharte, W. Stöcklein and A. Warsinke, Development of a creatinine ELISA and an amperometric antibody-based creatinine sensor with a detection limit in the nanomolar range *Anal. Chem.*, 2000, **72**, 916–921.