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## Data Article

## Synthesis of 1,2-phenylenediamine capturing molecule for the detection of diacetyl



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## ABSTRACT

Here we describe the design of 1,2-phenylenediamine capturing molecule and the synthesis steps necessary for its preparation. The designed 1,2-phenylenediamine derivative is able to capture diacetyl in solution, as shown by ESIMS, forming a chemical adduct, 1-4-quinoxaline. The methyl esters of diacetyl-adduct (DAA) and pentanedione-adduct (PDA) are incorporated to the lysines in BSA and the conjugate used for antibody screening and selection. In the research article is described an enzyme-linked immunosorbent assay developed to detect and quantify diacetyl in complex media.

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## Specifications Table

Subject area	Chemistry
More specific subject area	Chemical synthesis, assay development
Type of data	Text file, chemical structure
How data was acquired	ESIMS was recorded on a Bruker Esquire 3000-Plus Ion Trap instrument. MALDI-TOF-MS was recorded on Bruker Microflex instrument. HRESIMS was recorded on a Q-ToF Ultima instrument from Micromass. <sup>1</sup> H spectra were recorded at 20 °C on a Bruker 400 MHz instrument.

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Data format	Raw and analyzed data
Data accessibility	Data is with this article.
Related research article	L. Marri, A. M. Jansson, C. E. Christensen, O. Hindsgaul, <i>Analytical Biochemistry</i> , in press.

## Value of the Data

- The data explains the design of 1,2-phenylenediamine capturing molecule and could be used by others researchers.
- All the steps for the synthesis of the compounds here described and the methods can be followed by other researchers.
- The methods for incorporation of the diacetyl-adduct (DAA) and pentanedione-adduct (PDA) to the lysines in BSA can be used for preparation of other BSA-conjugates.

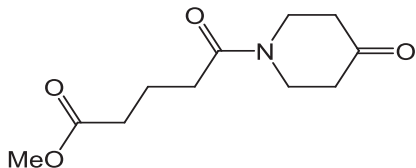
## 1. Data

The synthesis steps necessary for the preparation of the 1,2-phenylenediamine derivative (linker diamine) are described in details. The synthesis of the methyl esters of diacetyl-adduct (DAA) and pentanedione-adduct (PDA) and their incorporation in BSA to form the corresponding BSA-conjugates (BSA-DAA or BSA-PDA) are described.

## 2. Experimental design, materials and methods

### 2.1. General methods

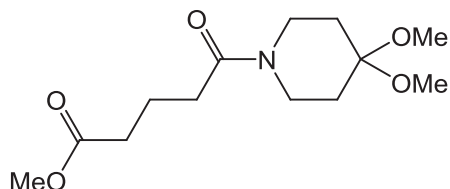
Organic solvents were concentrated under reduced pressure at  $< 40\text{ }^{\circ}\text{C}$ . Vacuum liquid chromatography was performed on silica gel (60 H, dried in oven at  $120\text{ }^{\circ}\text{C}$ ) Reversed phase chromatography was performed on Sep-Pak Plus  $\text{C}_{18}$  cartridge (Waters, washed with 10 mL MeCN, 10 mL 50% MeCN<sub>aq</sub> and 20 mL H<sub>2</sub>O before use). ESIMS was recorded on a Bruker Esquire 3000-Plus Ion Trap instrument with samples injected as solutions in MeCN-H<sub>2</sub>O 1:1 mixture. MALDI-TOF-MS was recorded on Bruker Microflex instrument, with sinapinic acid (10 mg/mL) in 30% MeCN in H<sub>2</sub>O containing 1% TFA, as matrix. HRESIMS was recorded on a Q-ToF Ultima instrument from Micromass. <sup>1</sup>H spectra were recorded at  $20\text{ }^{\circ}\text{C}$  on a Bruker 400 MHz instrument using CDCl<sub>3</sub> (7.27 ppm), CD<sub>3</sub>OD (3.31 ppm), CD<sub>3</sub>CN(1.94 ppm), or D<sub>2</sub>O (4.79 ppm) as internal standards.



### 1

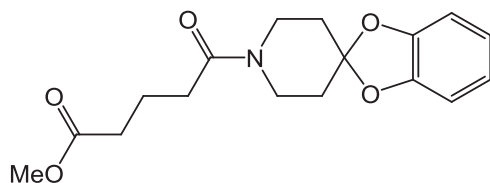
4-Piperidone hydrate hydrochloride (2.8 g, 0.018 mmol) and pyridine (8.5 g, 8.7 mL, 0.108 mmol) was dissolved in dichloromethane (100 mL) with help of sonication. A solution of glutaric acid monomethylester chloride (6.0 g, 0.036 mmol) in dichloromethane (20 mL) was slowly added, and the resulting solution was stirred for 20 h at  $20\text{ }^{\circ}\text{C}$ . The reaction mixture was extracted with 2 M HCl ( $1 \times 100\text{ mL}$ ), saturated NaHCO<sub>3</sub> ( $1 \times 100\text{ mL}$ ), brine ( $1 \times 100\text{ mL}$ ) and H<sub>2</sub>O ( $1 \times 100\text{ mL}$ ), followed by drying over NaSO<sub>4</sub>. Filtration, washing with dichloromethane and concentration gave **1** [1] (3.2 g, 78%, yellow oil).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm) 2.00 (dt, 2H), 2.43–2.51 (m, 8H), 3.68 (s, 3H), 3.77 (t, 2H,  $J = 6$  Hz), 3.88 (t, 2H,  $J = 6$  Hz). ESIMS calculated for  $\text{C}_{11}\text{H}_{17}\text{NO}_4$  227.1, found: 228.2  $[\text{M}+\text{H}]^+$ .



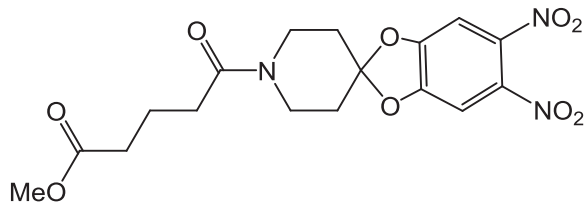
## 2

A mixture of **1** (0.45 g, 2.0 mmol), trimethyl orthoformate (0.24 g, 0.25 mL, 2.3 mmol), methanol (14 mL) and *p*-toluenesulfonic acid (4 mg) was gently heated while the methyl formate formed was distilled off through a short vigreux column. When no more methyl formate was distilled off, the reaction mixture was cooled, made basic with a few drops of 2N sodium methoxide and was partitioned between ethyl acetate (50 mL) and water (50 mL). The organic phase was washed with brine (50 mL) and dried over potassium carbonate. Filtration, washing with ethyl acetate and concentration afforded the dimethyl acetate **2** (0.45 g, 83%) [2]. The crude product was used in the following step without further purification.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm) 1.63–1.69 (m, 2H), 1.84–1.92 (dt, 2H,  $J = 7$  Hz), 2.29–2.35 (m, 4H), 3.13 (s, 6H), 3.35–3.40 (m, 2H), 3.50–3.55 (m, 2H), 3.60 (s, 3H). ESIMS calculated for  $\text{C}_{13}\text{H}_{23}\text{NO}_5$  273.2, found: 274.0  $[\text{M}+\text{H}]^+$ .



## 3

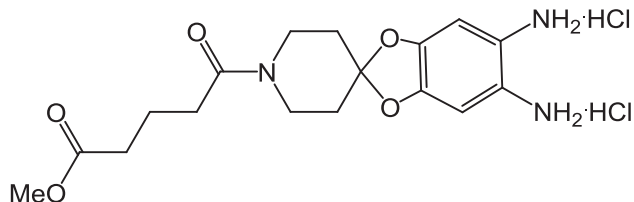
A mixture of the dimethyl acetal **2** (150 mg, 0.55 mmol), 1,2-dihydroxybenzene (72 mg, 0.65 mmol) and toluene (8 mL) was brought to reflux and part of the solvent (2 mL) was distilled through a short vigreux column. The temperature was lowered to 60 °C and *p*-toluenesulphonic acid (3 mg) was added. Distillation of the solvent was slowly continued until pure toluene was being collected. During this operation, addition of more toluene (8 mL) was necessary to prevent that the reaction mixture become dry. Triethylamine (0.1 mL) was added to the cooled reaction mixture, which was then partitioned between ethyl acetate and water. The organic phase was washed with water (1 × 20 mL), 0.1 M sodium hydroxide (2 × 20 mL), brine (2 × 20 mL) and dried over potassium carbonate. Filtration, washing with ethyl acetate and concentration gave **3** (120 mg, 69%) [2]. The crude product was used in the following step without further purification.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm) 1.88–1.97 (m, 6H), 2.32–2.44 (m, 4H), 3.57–3.62 (m, 2H), 3.61 (s, 3H), 3.72–3.77 (m, 2H). ESIMS calculated for  $\text{C}_{17}\text{H}_{21}\text{NO}_5$  319.1, found: 320.2  $[\text{M}+\text{H}]^+$ .



## 4

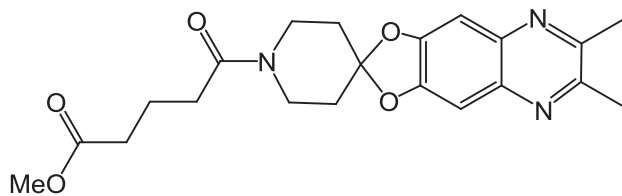
A solution of **3** (200 mg, 0.63 mmol) in acetic acid (1.5 mL) was added drop-wise under stirring into fuming nitric acid (2 mL) on an ice bath followed by stirring at room temperature for 1 h. The

reaction mixture was then poured into ice-water (5 mL) and the formed precipitate was isolated by centrifugation. The precipitate was washed with water ( $2 \times 2$  mL) and dried by freeze drying. This gave **4** (190 mg, 74%) [3]. The crude product was used in the following step without further purification.  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm) 1.89–1.98 (m, 2H), 2.01–2.10 (m, 4H), 2.36–2.44, (m, 4H), 3.61 (s, 3H), 7.23 (s, 2H). HRESIMS calculated for  $\text{C}_{17}\text{H}_{20}\text{N}_3\text{O}_9$  410.1200  $[\text{M}+\text{H}]^+$ , found: 410.1188.



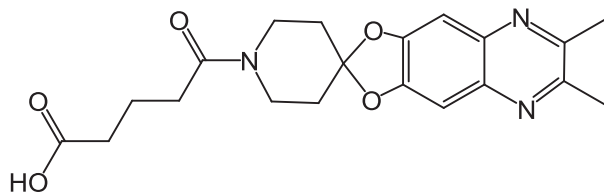
### 5

A mixture of di-nitro compound **4** (81 mg, 0.20 mmol), stannous chloride di-hydrate (226 mg, 1 mmol) and 12 M HCl (0.12 mL) in ethyl acetate (12 mL) was heated at 50 °C for 2 h. When adding additional 12 M HCl (0.12 mL) and ethyl acetate (20 mL) the product solidified and was isolated by centrifugation. The solid was washed with ethyl acetate ( $2 \times 10$  mL) and dried. This gave **5** (65 mg, 78%, yellow solid) [4,5]. The crude product was used in the following step without further purification.  $^1\text{H NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$  ppm) 1.77–1.86 (m, 2H), 1.86–1.91 (m, 2H), 1.94–1.99 (m, 2H), 2.32 (t, 2H,  $J = 7.2$ ), 2.41 (t, 2H,  $J = 7.4$ ) 3.60–3.70 (m, 4H), 6.58, (s, 2H). ESIMS calculated for  $\text{C}_{17}\text{H}_{23}\text{N}_3\text{O}_5$  349.2, found: 350.0  $[\text{M}+\text{H}]^+$ .



### 6

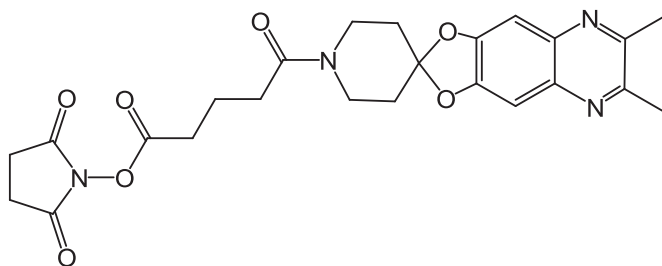
Compound **5** (10 mg, 0.024 mmol) and diacetyl (20 mg, 0.23 mol) was dissolved in water-acetic acid 2:1 (1 mL). The reaction mixture was stirred at room temperature for 1 hour. Dilution with 4 mL water followed by purification on SepPak (20–30% aq.  $\text{CH}_3\text{CN}$ ) gave the diacetyl adduct **6** (9 mg, 92%) [6].  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm) 1.74–1.81 (m,2H), 1.93–1.97 (m,2H), 2.00–2.04 (m,2H), 2.26–2.36 (m,4H), 2.51 (s,3H), 3.55 (s,3H), 3.57–3.61 (m,2H), 3.66–3.70 (m,2H), 7.10 (s,2H). ESIMS calculated for  $\text{C}_{21}\text{H}_{25}\text{N}_3\text{O}_5$  399.2, found: 400.1  $[\text{M}+\text{H}]^+$ .



### 7

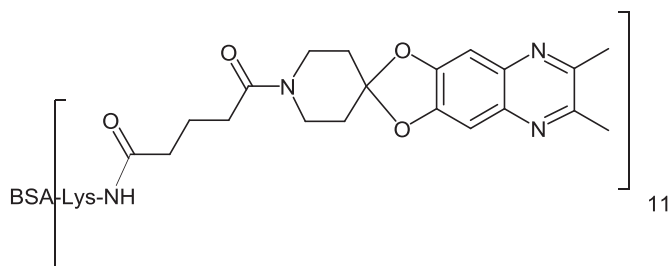
Compound **6** (9 mg, 0.022 mmol) was dissolved in a minimal volume of dioxane (0.2 mL). Water was added (0.3 mL) followed by 2 M sodium hydroxide (0.3 mL) and the reaction mixture was stirred at room temperature for 15 min. The pH was adjusted to 4.2 with 1 M and 0.1 M HCl. Purification was then directly performed by reversed phase chromatography on a  $\text{C}_{18}$  SepPak cartridge. After washing with water the product was eluted with water-acetonitrile 1:1. Evaporation of the solvents gave **7**

(8 mg, 95%).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$  ppm) 1.81–1.97 (m, 2H), 2.08–2.12 (m, 2H), 2.15–2.19 (m, 2H), 2.38 (t, 2H, 7.2 Hz), 2.54 (t, 2H, 7.4 Hz), 2.65 (s, 6H), 3.81–3.87 (m, 4H). HRESIMS calculated for  $\text{C}_{20}\text{H}_{23}\text{N}_3\text{O}_5$   $[\text{M}+\text{H}]^+$  386.1716, found: 386.1704.



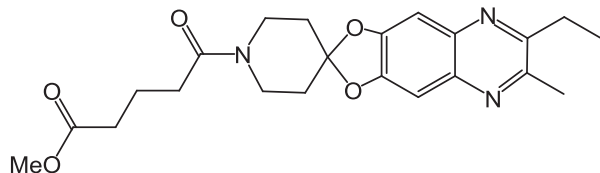
8

*N,N'*-disuccinimidyl carbonate (1.9 mg, 7.4  $\mu\text{mol}$ ) dissolved in dry DMF (0.1 mL) and 4-(dimethylamino)pyridine (1.5 mg, 12  $\mu\text{mol}$ ) dissolved in dry DMF (0.1 mL) were added to a solution of compound **7** (2 mg, 5.2  $\mu\text{mol}$ ) in dry DMF (0.4 mL) under magnetic stirring. Activation proceeded for 40 min. Dilution of reaction mixture with 0.01 M HCl to less than 10% DMF was followed by purification by reversed phase chromatography on a  $\text{C}_{18}$  SepPak cartridge. After washing with water-acetonitrile 9:1 and 8:1 the product was eluted with water-acetonitrile 1:1. Evaporation of the solvents gave **8** (2.2 mg, 88%) [7].  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm) 1.86–1.96 (m, 2H), 2.44 (t, 2H, 8 Hz), 2.51 (s, 6H), 2.63 (t, 2H, 7.5 Hz), 2.63 (s, 4H), 3.57–3.61 (m, 2H), 3.67–3.71 (m, 2H), 7.10 (s, 2H). ESIMS calculated for  $\text{C}_{24}\text{H}_{26}\text{N}_4\text{O}_7$  482.2, found: 483.1  $[\text{M}+\text{H}]^+$ .



9

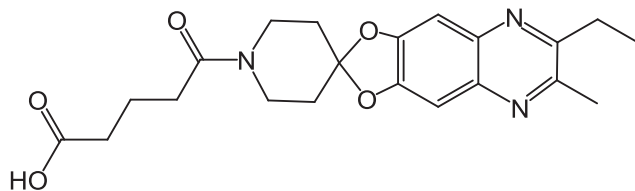
Bovine serum albumin (2 mg, 0.03  $\mu\text{mol}$ ) was dissolved in 0.1 M phosphate buffer pH 7.5 and added to a solution of compound **8** (0.9 mg, 2  $\mu\text{mol}$ ) in DMSO (100  $\mu\text{L}$ ) and 0.1 M phosphate buffer pH 7.5 (3 mL). The reaction mixture was gently vortexed overnight at room temperature and purified by concentration using a Millipore MW CO 30 000 filter (4000 rpm/6 min). The protein on the filter was washed with water ( $4 \times 4$  mL) and lyophilisation gave **9** [6]. MALDI-TOF-MS found  $m/z$  70 530, that corresponds to an incorporation of  $\sim 11$  adducts per BSA.



10

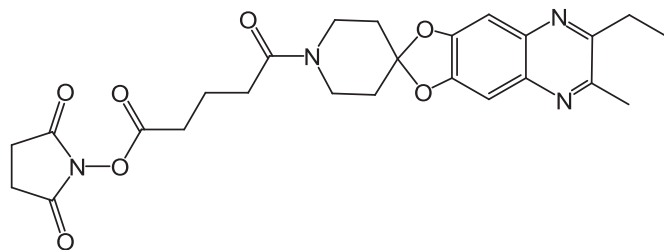
Compound **5** (35 mg, 0.083 mmol) and pentanedione (35 g,  $\mu\text{L}$ ) was dissolved in water-acetic acid 2:1 (2.5 mL). The reaction mixture was stirred at room temperature for 1 hour, and purification was then directly performed by reversed phase chromatography on a  $\text{C}_{18}$  SepPak cartridge. After washing with water, water-acetonitrile 9:1 and 8:2, the product was eluted with water-acetonitrile 1:1. Evaporation of the solvents gave **10** (27 mg, 79%) [6].  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm) 1.28 (t, 3H,

7.5 Hz), 1.95–2.07 (m, 6H), 2.30–2.47 (m, 4H), 2.65 (s, 3H), 2.90 (q, 2H, 7.5) 3.60 (s, 3H) 3.76–3.82 (m, 2H), 3.76–3.82 (m, 2H) 7.23 (s, 1H), 7.27 (s, 1H). ESIMS calculated for  $C_{22}H_{27}N_3O_5$  413.2, found: 414.1  $[M+H]^+$ .



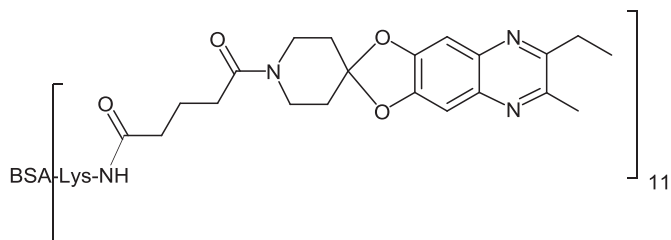
### 11

Compound **10** (7 mg, 0.017 mmol) was dissolved in a minimal volume of dioxane (0.2 mL). Water was added (0.3 mL) followed by 2 M sodium hydroxide (0.3 mL) and the reaction mixture was stirred at room temperature for 15 min. The pH was adjusted to 5 with 1 M and 0.1 M HCl. Purification was then directly performed by reversed phase chromatography on a  $C_{18}$  SepPak cartridge. After washing with water the product was eluted with water-acetonitrile 1:1. Evaporation of the solvents gave **11** (6.5 mg, 95%).  $^1H$  NMR (400 MHz,  $CDCl_3$ ,  $\delta$  ppm) 1.26 (t, 3H, 7 Hz), 1.78–1.86 (m, 2H), 1.98–2.02 (m, 2H), 2.07–2.11 (m, 2H), 2.16 (t, 2H, 6 Hz), 2.59 (s, 3H), 2.89 (q, 2H, 7 Hz), 3.73–3.77 (m, 4H), 7.10 (s, 1H), 7.15 (s, 1H).  $^1H$  NMR (800 MHz,  $CDCl_3$ ,  $\delta$ , ppm) 1.37 (t, 3H), 1.93, 2.11, 2.19 (m, 6H), 2.26, 2.53 (m, 4H), 2.69 (2, 3H), 2.99 (q, 2H) 3.70 (s, 3H) 3.86 (m, 4H), 7.20 (s, 1H), 7.25 (s, 1H). HRESIMS calculated for  $C_{21}H_{25}N_3O_5$   $[M+H]^+$  400.1873, found: 400.1848.



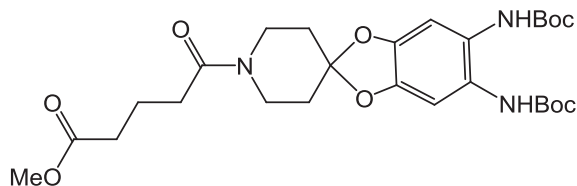
### 12

*N,N'*-disuccinimidyl carbonate (3.3 mg, 13  $\mu$ mol) dissolved in dry DMF (0.1 mL) and 4-(dimethylamino)pyridine (3.3 mg, 27  $\mu$ mol) dissolved in dry DMF (0.1 mL) were added to a solution of compound **11** (3.25 mg, 8  $\mu$ mol) in dry DMF (0.1 mL) under magnetic stirring. Activation proceeded for 40 min. Dilution of reaction mixture with 0.01 M HCl to less than 10% DMF was followed by purification by reversed phase chromatography on a  $C_{18}$  SepPak cartridge. After washing with water-acetonitrile 9:1 and 8:1 the product was eluted with water-acetonitrile 1:1. Evaporation of the solvents gave **12** (3.5 mg, 87%) [7].  $^1H$  NMR (400 MHz,  $CDCl_3$ ,  $\delta$  ppm) 1.22 (t, 3H, 8 Hz), 1.85–1.95 (m, 2H), 1.91–2.02 (m, 4H), 2.43 (t, 2H, 8 Hz), 2.52 (s, 3H), 2.62 (m, 2H, 8 Hz), 2.67 (s, 4H), 2.84 (q, 2H, 8 Hz), 3.56–3.61 (m, 2H), 3.66–3.71 (m, 2H), 7.09 (s, 1H), 7.10 (s, 1H). ESIMS calculated for  $C_{25}H_{28}N_4O_7$  496.2, found: 497.1  $[M+H]^+$ .

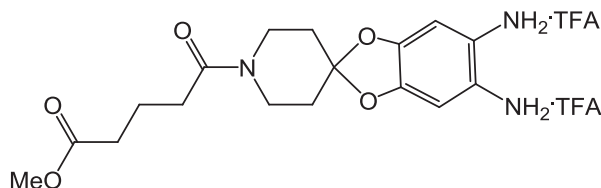


### 13

Bovine serum albumin (3.8 mg,  $x \mu\text{mol}$ ) was dissolved in 0.1 M phosphate buffer pH 7.5 (1.6 mL) and added to a solution of compound **12** (3.5 mg,  $7 \mu\text{mol}$ ) in DMSO (200  $\mu\text{L}$ ) and 0.1 M phosphate buffer pH 7.5 (0.8 mL). The reaction mixture was gently “shaken” overnight and purified by concentration using a Millipore MW CO 30 000 filter (4000 rpm/6 min). The residual protein on the filter was washed with water ( $4 \times 4 \text{ mL}$ ) and lyophilisation gave **13** [6]. MALDI-TOF-MS found  $m/z$  70 380, that corresponds to an incorporation of  $\sim 11$  adducts per BSA.

**14**

To a mixture of **5** (7 mg,  $17 \mu\text{mol}$ ) in dioxane (300  $\mu\text{L}$ ) and water (100  $\mu\text{L}$ ), under argon, was added triethylamine (11.5  $\mu\text{L}$ ,  $83 \mu\text{mol}$ ) followed by di-*t*-butyl dicarbonate (11 mg,  $50 \mu\text{mol}$ ). The reaction mixture was stirred at room temperature for 3 h. The solvents were evaporated and the residue, dissolved in a minimal volume EtOAc, was purified by VLC (silica 60H). The product was eluted with 60–70% EtOAc on hexane. This gave **14** (7 mg, 67%).  $^1\text{H NMR}$  (400 MHz,  $\text{CD}_3\text{CN}$  ppm) 1.40 (s, 18 H), 1.80–1.90 (m, 2H), 1.87–2.05 (m, 4H), 2.30–2.45 (m, 4H), 3.58–3.74 (m, 7H), 6.98 (s, 2H). ESIMS calculated for  $\text{C}_{27}\text{H}_{28}\text{N}_4\text{O}_7$  549.3, found: 550.1  $[\text{M}+\text{H}]^+$ , 572.1  $[\text{M}+\text{Na}]^+$ , 588.1  $[\text{M}+\text{K}]^+$  (Fig. 3) [8].

**15**

Compound **14** (0.1 mg,  $0.2 \mu\text{mol}$ ) was stirred with 10%  $\text{CH}_2\text{Cl}_2$  in TFA (70  $\mu\text{L}$ ) under argon for 10 min. Solvents were evaporated under a stream of argon followed by 5 min in speed-vac. The TFA-salt of the linker phenylenediamine, **15**, was directly used in capture reactions. ESIMS calculated for  $\text{C}_{17}\text{H}_{23}\text{N}_3\text{O}_5$  349.2, found: 350.2  $[\text{M}+\text{H}]^+$ , 372.1  $[\text{M}+\text{Na}]^+$ , 721.3  $[2\text{M}+\text{Na}]^+$  (Fig. 3) [8].

Reaction of compound **15** with diacetyl to afford diacetyl adduct **6**, analysed with ESIMS.

To a solution of compound **15** ( $\sim 0.2 \mu\text{mol}$ ) in water (20  $\mu\text{L}$ ) was added 10  $\mu\text{L}$  of a solution of diacetyl in water (0.5 mg/100  $\mu\text{L}$ ). ESIMS after 2 min (dilution  $\times 10$  with  $\text{MeCN}-\text{H}_2\text{O}$  1:1) shows the diacetyl adduct product. ESIMS calculated for  $\text{C}_{21}\text{H}_{25}\text{N}_3\text{O}_5$  399.2, found: 400.2  $[\text{M}+\text{H}]^+$  (Fig. 3) [8].

## Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2017.09.072>.

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