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# Synthesis of Dopamine and Serotonin Derivatives for Immobilization on a Solid Support

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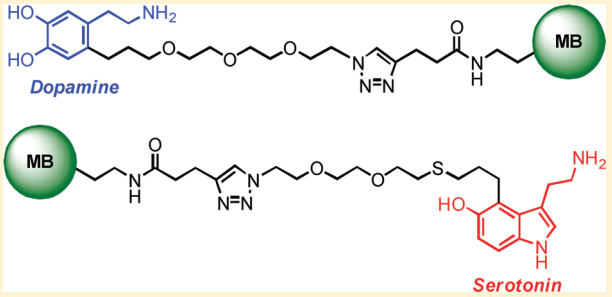
Supporting Information

**ABSTRACT:** The two important neurotransmitters dopamine and serotonin are synthesized with short PEG tethers and immobilized on a magnetic solid support. The tether is attached to the aromatic moiety of the neurotransmitters to conserve their original functional groups. This approach causes minimal alteration of the original structure with the aim of optimizing the immobilized neurotransmitters for aptamer selection by SELEX. For the dopamine derivative, the tether is attached to the aromatic core of a dopamine precursor by the Sonogashira reaction. For serotonin, a link to the indole core is introduced by a Claisen rearrangement from the allylated phenol moiety of serotonin. The tethers are azide-functionalized, which enables coupling to alkyne-modified magnetic beads. The coupling to the magnetic beads is quantified by UV spectroscopy using Fmoc-monitoring of the immobilized dopamine and serotonin derivatives.




## INTRODUCTION

Dopamine and serotonin are essential neurotransmitters controlling important aspects of human behavior. Imbalance in the regulation of neurotransmitters plays a central role in many major neurological diseases, for example, Parkinson's disease, schizophrenia, and depression.[1] A variety of methods have been developed to detect neurotransmitters in vivo, such as microdialysis, fast-scan cyclic voltammetry, and positron emission tomography.[2,3] However, many of these methods suffer from lack of specificity or modest temporal resolution. An alternative method to detect neurotransmitters may be aptamer-based detection, which can be extended to both an optical or electrochemical readout.[4−8] Here, we present the design and synthesis of new dopamine and serotonin derivatives optimized for the selection of aptamers that, in turn, will be used for electrochemical detection of the two neurotransmitters.

Aptamers are synthetic oligonucleotides or peptides that are selected in vitro for binding to a specific target, hereby representing an attractive alternative to antibodies.[4] Oligonucleotide aptamers are selected from a large library of oligonucleotides for binding to a ligand by repeated selection and amplification, a process called systematic evolution of ligands by exponential enrichment (SELEX).[9,10] The selected aptamers bind to their target by forming a unique secondary and tertiary structure with high specificity for the target. Aptamers have been selected for binding to a variety of different molecular targets ranging from small organic molecules to proteins or even cell surfaces.[4] In the majority of reported aptamer selections, the ligands are immobilized on a solid support, which, in the selection process, simplifies the removal of nonbinding oligonucleotides.

While aptamers for serotonin have not yet been reported, Mannironi et al.[11] have described the selection of an RNA aptamer for dopamine. The aptamer binds to dopamine with a $K_d$ of 2.8 $\mu$M. The specificity of the aptamer was albeit low compared to the binding of dopamine as it showed 58, 30, and 23% binding of norepinephrine, L-DOPA, and catechol, respectively. We speculated that the relatively low specificity of the aptamer originates from the dopamine immobilization strategy used for the aptamer selection. Dopamine was linked to the solid support via an amide bond (Figure 1). Since the amide group is uncharged and has a different geometry than the primary amine in dopamine, which is protonated at physiological pH, the dopamine aptamer was primarily selected for binding to the catechol moiety of dopamine.

The goal of the current study is to synthesize new dopamine and serotonin derivatives for immobilization on solid supports as improved targets for selection of aptamers by the SELEX procedure. Prior to this study, all examples of dopamine and serotonin immobilization onto a solid support have been via the primary amine.[11−14] In the case of serotonin, the structurally closely related 5-hydroxytryptophan was immobilized via the carboxylic acid group, thereby conserving the amino group in the immobilized species (Figure 1).[15]

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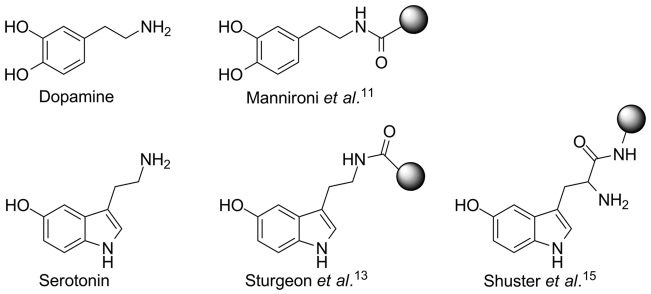


**Figure 1.** Structures of dopamine and serotonin as well as selected previous examples on immobilization of dopamine,[11] serotonin,[13] and the structurally related 5-hydroxytryptophan.[15]

The previously reported methods share the disadvantage of disrupting the electronic properties of the original primary amine of the neurotransmitters. The immobilized derivates may, therefore, not be optimal as targets in aptamer selection. Here, we present new approaches for immobilization of the two neurotransmitters in which the functional groups of native dopamine and serotonin are conserved and instead a polyethylene glycol (PEG) linker with a terminal azide has been connected to the aryl skeleton of the neurotransmitters. The two target derivatives of dopamine (**1**) and serotonin (**2**), presented in Figure 2, have three important characteristics in

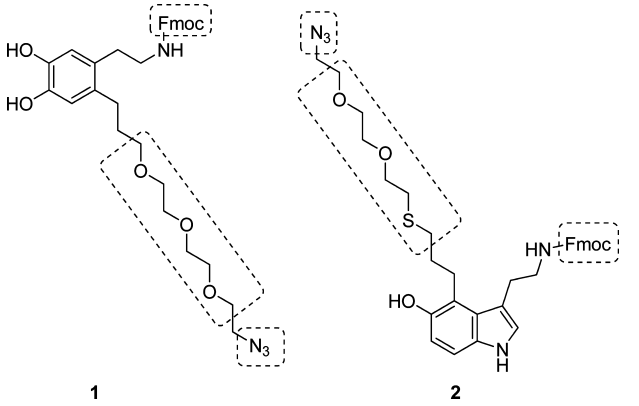


**Figure 2.** Synthetic targets presented for dopamine analogue **1** and serotonin analogue **2**.

common. Attachment to the solid support is achieved through a PEG linker. The PEG linker is chemically inert, water-soluble, and known to reduce nonspecific binding to biomolecules.[16,17] The immobilization onto the solid support proceeds via an azide group situated at the terminal end of the PEG linker. By the copper-catalyzed Huisgen–Meldal–Sharpless 1,3-dipolar cycloaddition reaction between a terminal alkyne and an azide, it is possible to react **1** and **2** with an alkyne-modified solid support in an efficient and chemoselective manner.[18,19] Furthermore, an Fmoc group has been introduced to function as an indicator of immobilization efficiency. This can be measured by cleaving the Fmoc group after immobilization. The liberated 9-methylidenefluorene is detected by UV spectroscopy and enables the facile quantification of **1** and **2** on the solid support.

## RESULTS AND DISCUSSION

We decided to pursue the synthesis of dopamine derivative **1** (Figure 2), for the following two reasons: (i) it has the linker attached to the aromatic moiety, causing minimal interference with the catechol and ethyleneamine moieties, and (ii) a useful 2-bromoaryl derivative **3** was readily available (Scheme 1).

**Synthesis of Dopamine Derivative 1.** The synthesis was initiated by subjecting the benzylic position of **3** (Scheme 1) to an $S_N2$ attack from cyanide in DMF, affording **4** in an excellent yield of 92%. The electron-rich nitrile compound was coupled with propargylic alcohol by a Sonogashira cross-coupling, giving **5** in a poor yield of 27%. We have not been able to optimize this step further, which mainly is accredited to the difficulty of palladium(0) to initiate the catalytic cycle by oxidative addition. Furthermore, the benzylic nitrile group can potentially coordinate palladium in a cyclic system, hereby retarding the efficiency of the catalyst.

The propargylic alcohol moiety was then hydrogenated to the aliphatic alcohol **6**, using hydrogen gas and Pd/C at atmospheric pressure. Notably, the nitrile group was stable under these conditions. The primary alcohol was protected as the acetyl ester **7**, after which the nitrile group was reduced to the amine and protected as the *tert*-butyl carbamate in a one-pot nickel(II)-catalyzed reaction, yielding **8**.[20] The acetyl protecting group was removed by methoxide, resulting in the free primary alcohol **9**. Reactions **5**–**9** proceeded according to TLC and NMR analysis with full conversion. As a result, the reactions only required aqueous work up and a final purification step via flash column chromatography. The four steps resulted in an overall yield of 48%, that is, an average yield of 83% per step.

To introduce the PEG-azide linker, the primary alcohol was mesylated using MsCl and triethylamine in dry diethyl ether, giving **10** in a quantitative yield. Next, PEG-azide linker **11** was treated with sodium hydride in DMF to generate the sodium alkoxide salt. Subsequent slow addition of **10** facilitated the crucial $S_N2$ reaction, giving **12** in a 55% yield.

At this stage of the synthesis, it would be possible to convert **12** to the globally deprotected molecule; however, since our intention was to install the Fmoc as a reporter group for quantification of the subsequent immobilization, the Boc group was cleaved using 4 M HCl in dioxane. The resulting hydrochloric salt was subjected to Fmoc-protection conditions, giving **13** in a yield of 60% over two steps. Thereafter, only the acetal protection group was to be removed. However, this turned out to be more troublesome than anticipated. Treatment with hydrochloric acid in various concentrations and at different temperatures, or use of different Lewis acids, such as $BBr_3$ and $BCl_3$, turned out to be unsuccessful. Either the acetal remained stable or the PEG linker was degraded.[21] Eventually, the solution was to transform the stable acetal into the much more labile *ortho*-ester.[22] This was performed by reacting **13** with $Pb(OAc)_4$ in benzene, giving **14**, which could undergo acidic hydrolysis, generating the desired title compound **1** in 30% yield over two steps.

**Synthesis of Serotonin Derivative 2.** For the synthesis of a suitable serotonin derivative, we decided to start from serotonin and introduce the linker to the 4-position of the indole skeleton. A report from Macor et al.[23] demonstrated that an allyl group can be introduced in this position in high yields via a Claisen rearrangement of the allylated phenol moiety (Scheme 2).

First, the primary amine in serotonin hydrochloride **15** was Boc-protected in 93% yield, followed by O-allylation of **16** using allyl iodide and $Cs_2CO_3$ in acetone to give **17** in 74% yield (Scheme 2). Hereby, the stage was set for a central


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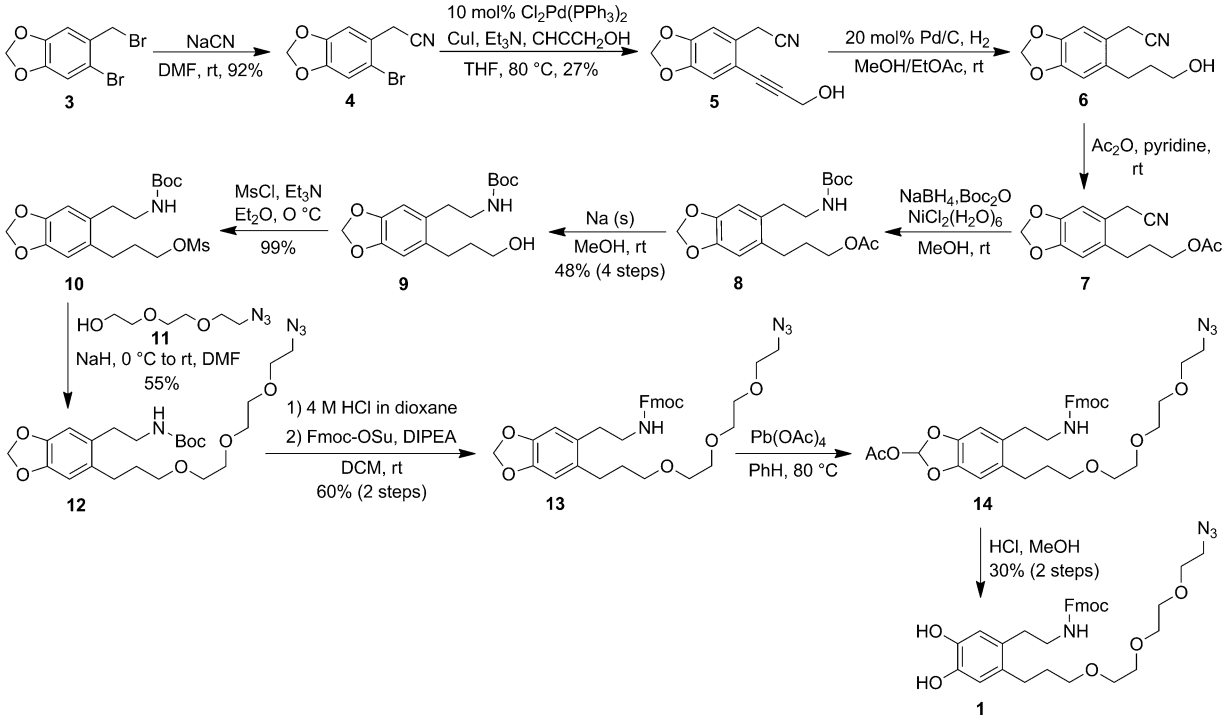
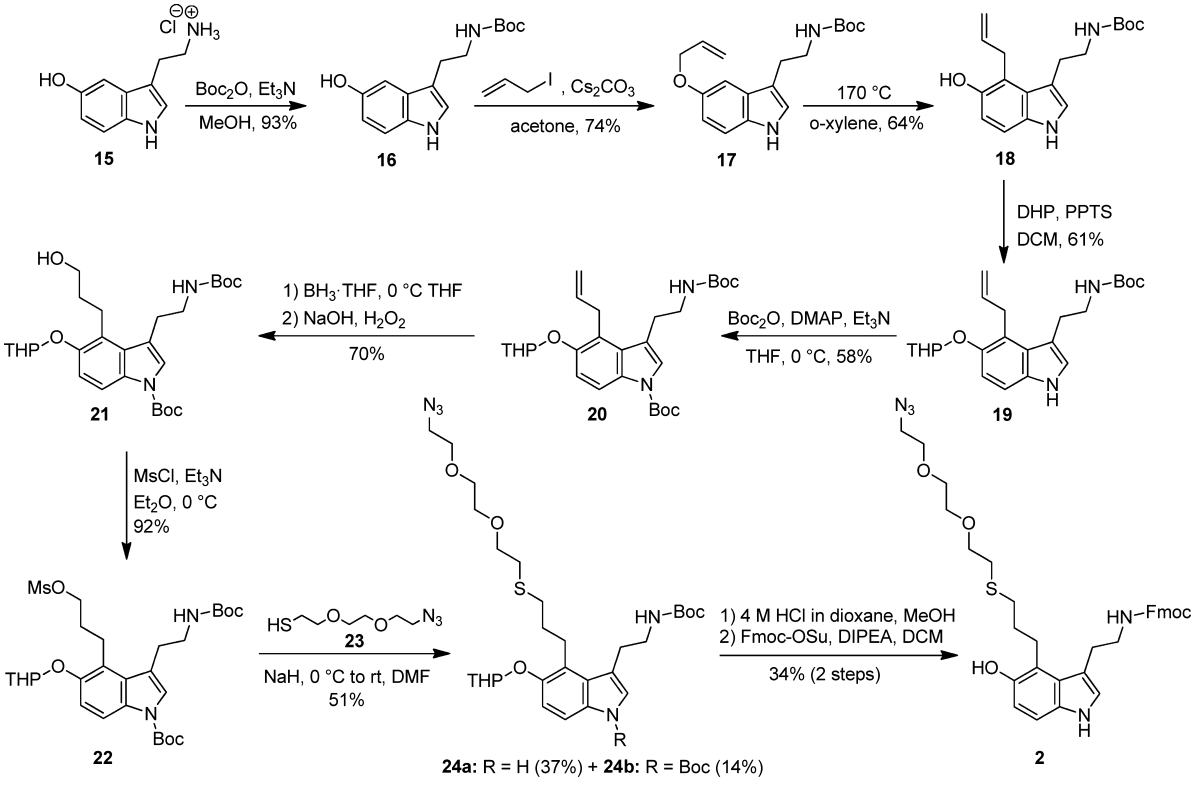
**Scheme 1. Synthesis of the PEG-Azide Dopamine Derivative**




**Scheme 2. Synthesis of the PEG-Azide Serotonin Derivative**




Claisen rearrangement in which a new carbon−carbon bond to the indole moiety was formed and later utilized in the tether to the solid support. The [3,3]-sigmatropic rearrangement proceeded with full regioselectivity to form the 4-allylindole derivative **18** in a satisfying yield of 64%. The liberated phenol was then protected to avoid O-alkylation in subsequent steps. A variety of protective groups were examined for protection of the phenol. A screening showed that protection with *tert*-butyl carbonate, TIPS, TBDPS, and MOM were either unsuccessful, irreproducible, or the protected species was labile in the subsequent alkylation reaction. Fortunately, the application of THP as protection of the phenol was successful. The THP ether was formed by using 3,4-dihydro-2*H*-pyran and PPTS in dry DCM, giving **19** in 61% yield. The indole nitrogen has an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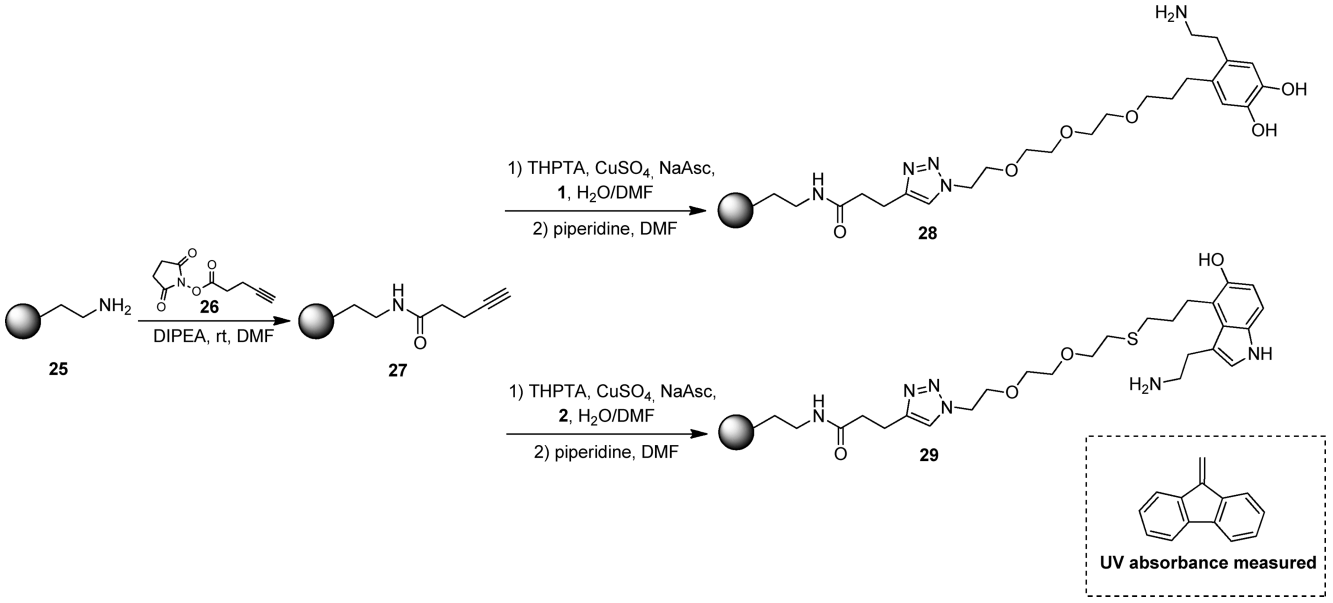


**Figure 3.** Immobilization of neurotransmitter derivatives **1** and **2** on an alkyne-modified solid support.

acidity comparably to that of an aliphatic alcohol. As a consequence, the indole nitrogen was Boc-protected to avoid unwanted side reactions. After Boc protection, **20** was obtained in 58% yield.

Hydroboration of the terminal alkene in **20**, followed by oxidation, resulted in the primary alcohol **21** in 70% yield with complete regioselectivity. The alcohol of **21** was converted into an electrophile by mesylation to give **22** in 92% yield.

We attempted to react **22** with **11** to connect the PEG linker to the serotonin skeleton via an ether, in analogy to the synthesis of the dopamine derivative **1** (Scheme 1). However, very poor yields were obtained from several attempts to perform this reaction (<5%), and therefore, we speculated that better results may be obtained by using a thiol nucleophile instead of the alcohol **11**. To test this, the thiol- and azide-functionalized PEG linker **23** was prepared in four steps (see the Experimental Section). Thiol **23** was coupled to the serotonin skeleton by deprotonation of the thiol, followed by substitution with the mesylate, to give **24a** and **24b** in a total yield of 51%.

All acid labile protecting groups in **24a** and **24b** were removed in a one-pot global deprotection by treatment with methanol and 4 M HCl in dioxane. In the final step, 9-fluorenylmethyl-*N*-succinimidyl carbonate and diisopropylamine were used to Fmoc-protect the amine, giving the final target molecule **2** in 34% yield over two steps.

**Immobilization.** For immobilization of the two linker-functionalized neurotransmitter derivatives commercially available, amino-terminated magnetic beads were chosen. Such magnetic beads are commonly used in the SELEX process[24,25] because of their intrinsic magnetic properties, which ease subsequent manipulations. To obtain an alkyne-modified solid support, the amino-modified magnetic beads were reacted with an alkyne NHS ester[26] (Figure 3). The reaction between an NHS-activated acid and an amine is normally a very high yielding reaction; however, to functionalize as many amines as possible, the reaction was repeated a total of three times. Any remaining amines were capped as the acetyl ester using acetic anhydride and diisopropylethylamine. Consequently, alkyne-modified magnetic beads were obtained and set for further modification.

The synthesized neurotransmitter derivatives were linked to the alkyne-modified magnetic beads via the copper-catalyzed Huisgen−Meldal−Sharpless 1,3 dipolar cycloaddition by utilizing $CuSO_4$, tris-(1-[3-hydroxypropyl]triazolyl-4-methyl)-amine (THPTA),[27] and (+)-sodium-L-ascorbate. As a result, the neurotransmitter derivatives were linked to the solid support by a 1,4-disubstituted 1,2,3-triazole, giving rise to a solid support functionalized with dopamine derivative **1** and a solid support functionalized with serotonin derivative **2**. It was investigated whether the catechol and 5-hydroxyindole, present in the dopamine and serotonin derivatives, are stable under the conditions applied for the click reactions. Dopamine and serotonin were separately exposed to the click reaction conditions, and subsequently, the product was analyzed by $^1$H NMR. It was found that the groups prone to oxidation were stable to the experimental conditions (Supporting Information). Therefore, we assume that the derivatives **1** and **2** are also stable under the applied conditions.

To enable quantification of the amount of neurotransmitter derivatives immobilized on the solid support, the Fmoc group was cleaved and the UV ($\lambda$ = 301 nm) absorption was measured. Significant absorption by 9-methylidenefluorene was measured, and an estimate of the amount of immobilized neurotransmitter derivatives onto the solid support was calculated by the Lambert−Beer law. Accordingly, a dopamine-functionalized solid support (30 nmol loading/8 × $10^8$ beads) and a serotonin-functionalized solid support (29 nmol loading/9 × $10^8$ beads) were obtained.

The beads **28** and **29**, containing the immobilized neurotransmitters, were stored below 0 °C in a suspension containing ascorbic acid as antioxidant to avoid oxidation of the neurotransmitters.

## ■ CONCLUSION

PEG-azide derivatives of dopamine (12 steps) and serotonin (10 steps) have been synthesized and successfully immobilized onto a magnetic solid support via the Huisgen−Meldal−Sharpless click reaction. Furthermore, the incorporated Fmoc


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reporter group enabled the facile quantification of the neurotransmitter derivatives immobilized onto the solid support. Application of the immobilized neurotransmitters described here for selection of aptamers toward serotonin and dopamine is currently in progress.

## EXPERIMENTAL SECTION

**General Experimental Details.** For all water- and/or oxygen-sensitive reactions, flame-dried glassware, which was purged with argon and evacuated a total of three times, was used. All reactions were monitored by thin-layer chromatography (TLC) analysis on Merck silica gel 60 $F_{254}$ TLC plates. The TLC plates were visualized by exposure to UV (254 nm) or by staining with either a basic aq. $KMnO_4$ solution or a cerium−ammonium−molybdate stain. Flash column chromatography was performed using Merck silica gel 60 (230−400 mesh). All solvents were of HPLC grade quality. For inert reactions, the solvents THF and DCM were dried prior to use by the use of a solvent purification system. Diethyl ether was dried with sodium, and triethylamine was distilled from $CaH_2$ before use. Unless noted, all used reagents are commercially available. The reagents were purchased at the highest possible quality and used without further purification.

NMR spectra were recorded at 400 MHz ($^1$H NMR) and at 100 MHz ($^{13}$C NMR) and calibrated to the residual solvent peak. Chemical shifts are reported in parts per million and coupling constants are reported in hertz. In the interpretation of the $^1$H NMR spectra, the following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; qui, quintet; sext, sextet; m, multiplet; br, broad. Melting points are uncorrected. High-resolution mass spectrometry (HRMS) was conducted using electrospray ionization.

Compound **3** (CAS: 5434-47-9) was purchased from a chemical supplier. Compound **11** is commercially available; however, it was synthesized according to a literature procedure.[28] Compound **15** (CAS: 153-98-0) was purchased from a chemical supplier, as were the magnetic beads, Dynabeads M-270 amine.

**2-(6-Bromobenzo[*d*][1,3]dioxol-5-yl)acetonitrile (4).**[29] To a 250 mL flame-dried flask under an argon atmosphere was added **3** (10.20 g, 34.70 mmol), which was dissolved in dry DMF. Thereafter, sodium cyanide (6.82 g, 139 mmol) was added portionwise, and the reaction was left to stir for 3 h.

The reaction was quenched by the addition of water and then extracted three times with EtOAc. The combined organic fractions were thoroughly washed with water, dried ($MgSO_4$), filtered under suction, and evaporated. The resulting yellow compound was purified by flash chromatography (pentane/EtOAc 1:1). The product was isolated as a white solid (7.5 g, 32.3 mmol, 92% yield).

mp(uncorr.) 61.8−66.1 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.01 (s, 1H), 6.95 (s, 1H), 6.00 (s, 2H), 3.72 (s, 2H).$^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.5, 148.0, 122.7, 117.2, 114.3, 113.0, 109.5, 102.3, 24.7. HRMS (ES) $m/z$ $[M + Na]^+$ calcd for $C_9H_6BrNO_2Na$: 261.9480. Found: 261.9484.

**2-(6-(3-Hydroxyprop-1-yn-1-yl)benzo[*d*][1,3]dioxol-5-yl)-acetonitrile (5).** To a flame-dried Schlenk flask containing an argon atmosphere was added **4** (4.070 g, 16.94 mmol), which was dissolved in freshly distilled THF and $Et_3N$ (1:1). Propargyl alcohol (3.9 mL, 68 mmol) was added, and the mixture was degassed with argon for 30 min, after which $Cl_2Pd(II)(PPh_3)_2$ (1.19 g, 1.69 mmol) and CuI (0.161 g, 0.845 mmol) were added. The resulting yellow mixture was heated to 80 °C and left to stir overnight.

The black mixture was filtered through Celite and evaporated to dryness. Thereafter, the resulting yellow oil was purified by flash column chromatography (pentane/EtOAc 1:1), and the product was isolated as a white solid (1.00 g, 4.65 mmol, 27%).

mp(uncorr.) 133.2−134.3 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 6.92 (s, 1H), 6.91 (s, 1H), 6.02 (s, 2H), 4.53 (d, $J$ = 6.2, 2H), 3.82 (s, 2H), 1.76 (t, $J$ = 6.2, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.9, 147.5, 126.7, 117.7, 115.4, 112.1, 108.9, 102.1, 92.4, 82.6, 51.6, 22.7. HRMS (ES) $m/z$ $[M + Na]^+$ calcd for $C_{12}H_9NO_3Na$: 238.0480. Found: 238.0491.

**2-(6-(3-Hydroxypropyl)benzo[*d*][1,3]dioxol-5-yl)acetonitrile (6).** In a 100 mL Schlenk flask, **5** (0.660 g, 3.07 mmol) was dissolved in MeOH (10 mL) and EtOAc (10 mL). To the resulting mixture was added Pd/C (65 mg, 0.55 mmol) from a batch containing a 10% w/w loading. Subsequently, the reaction was purged with hydrogen gas under vigorous magnetic stirring.

After reacting overnight, the reaction was filtered through Celite and evaporated to dryness in vacuo, affording crude **6** as a yellow oil (100% conversion).

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 6.81 (s, 1H), 6.69 (s, 1H), 5.93 (s, 2H), 3.81−3.45 (m, 4H), 2.74−2.47 (m, 2H), 2.06 (brs, 1H), 1.93−1.65 (m, 2H).$^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 147.8, 146.4, 133.6, 120.8, 118.4, 109.8, 109.2, 101.4, 61.5, 33.4, 28.7, 21.2. HRMS (ES) $m/z$ $[M + Na]^+$ calcd for $C_{12}H_{13}NO_3Na$: 242.0793. Found: 242.0788.

**3-(6-(Cyanomethyl)benzo[*d*][1,3]dioxol-5-yl)propyl acetate (7).** To a 250 mL flask was added crude **6** (0.650 g, 2.96 mmol), which was dissolved in pyridine (30 mL) and acetic anhydride (30 mL). The reaction was left to react overnight, after which the reaction was quenched with 0.1 M hydrochloric acid (20 mL). The mixture was transferred to a 250 mL separation funnel and then extracted with EtOAc (3 × 60 mL). The combined organic fractions were washed with $NaHCO_3$ (2 × 50 mL), dried ($MgSO_4$), filtered under suction, and evaporated to dryness to afford crude **7** as a yellow oil (100% conversion).

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 6.78 (s, 1H), 6.64 (s, 1H), 5.89 (s, 2H), 4.05 (t, $J$ = 6.3, 2H), 3.58 (s, 2H), 2.65−2.48 (m, 2H), 2.02 (s, 3H), 1.93−1.75 (m, 2H).$^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 171.1, 147.9, 146.5, 132.8, 120.7, 118.0, 109.7, 109.4, 101.4, 63.5, 29.4, 28.9, 21.2, 21.0. HRMS (ES) $m/z$ $[M + Na]^+$ calcd for $C_{14}H_{15}NO_4Na$: 284.0899. Found: 284.0898.

**3-(6-(2-((*tert*-Butoxycarbonyl)amino)ethyl)benzo[*d*][1,3]-dioxol-5-yl)propyl Acetate (8).** A solution of crude **7** (0.778 g, 2.98 mmol) in MeOH (25 mL) was cooled to 0 °C. Thereafter, $Boc_2O$ (1.36 g, 6.23 mmol) and $NiCl_2(H_2O)_6$ (0.100 g, 0.42 mmol) were added. After 5 min of stirring, $NaBH_4$ (0.788 g, 20.8 mmol) was added portionwise, and the black solution was left to stir for 3 h, whereupon $NaBH_4$ (0.788 g, 20.8 mmol) again was added. Subsequently, the reaction was left to react overnight.

The resulting white solution was diluted with EtOAc, washed twice with $NaHCO_3$, dried ($MgSO_4$), filtered under suction, and evaporated to dryness in vacuo, furnishing crude **8** (100% conversion).

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 6.60 (s, 1H), 6.59 (s, 1H), 5.84 (s, 2H), 4.72 (brs, 1H), 4.04 (t, $J$ = 6.5, 2H), 3.37−3.10 (m, 2H), 2.67 (t, $J$ = 7.2, 2H), 2.64−2.49 (m, 2H), 2.02 (s, 3H), 1.87−1.76 (m, 2H), 1.39 (s, 9H).$^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 171.3, 156.0, 146.3, 146.1, 132.8, 129.8, 109.7, 109.5, 100.9, 79.3, 63.9, 41.7, 32.9, 30.3, 28.8, 28.5, 21.1. HRMS (ES) $m/z$ $[M + Na]^+$ calcd for $C_{19}H_{27}NO_6Na$: 388.1736. Found: 388.1731.

***tert*-Butyl(2-(6-(3-hydroxypropyl)benzo[*d*][1,3]dioxol-5-yl)-ethyl)carbamate (9).** To MeOH (10 mL) in a 25 mL flask was added Na (0.050 g, 2.0 mmol). The freshly generated solution of MeONa/MeOH was then added directly to a solution of crude **8** (1.04 g, 2.84 mmol) in MeOH (15 mL), and the reaction was left to stir overnight at room temperature.

The reaction was evaporated to dryness and purified via flash column chromatography (pentane/EtOAc 6:4), affording pure **9** (0.482 g, 1.49 mmol, 48% after four steps).

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 6.65 (s, 1H), 6.60 (s, 1H), 5.87 (s, 2H), 4.86 (brs, 1H), 3.66 (t, $J$ = 5.6, 2H), 3.23 (q, $J$ = 7.6, 2H), 2.72 (t, $J$ = 7.6, 2H), 2.67 (t, $J$ = 8.0, 2H), 2.16 (brs, 1H), 1.85−1.70 (m, 2H), 1.42 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 156.3, 146.2, 145.8, 133.8, 129.6, 109.7, 109.7, 100.8, 79.6, 62.1, 42.0, 34.8, 33.3, 28.9, 28.5. HRMS (ES) $m/z$ $[M + Na]^+$ calcd for $C_{17}H_{25}NO_5Na$: 346.1630. Found: 346.1609.

**3-(6-(2-((*tert*-Butoxycarbonyl)amino)ethyl)benzo[*d*][1,3]-dioxol-5-yl)propyl Methanesulfonate (10).** To a solution of **9** (0.482 g, 1.49 mmol) in dry diethyl ether was added $Et_3N$ (0.41 mL, 3.0 mmol). Thereafter, the reaction mixture was cooled to 0 °C, followed by the addition of MsCl (0.17 mL, 2.2 mmol). The reaction was left to stir for 2 h, after which the resulting slurry was diluted with


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EtOAc and washed with water (×2) and brine. The combined water and brine fraction was extracted once with EtOAc. Subsequently, the combined organic fractions were dried ($MgSO_4$), filtered under suction, and evaporated to dryness.

The resulting yellow oil was purified via flash column chromatography (pentane/EtOAc 13:7), affording **10** (590 mg, 1.47 mmol, 99%) as a white yellow solid.

mp(uncorr.) 90.0−91.0 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 6.60 (m, 2H), 5.85 (s, 2H), 4.77 (brs, 1H), 4.21 (t, $J$ = 6.2, 2H), 3.21 (q, $J$ = 8.0, 2H), 3.00 (s, 3H), 2.73−2.57 (m, 4H), 2.02−1.84 (m, 2H), 1.39 (s, 9H).$^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 155.8, 146.2, 146.0, 131.8, 129.8, 109.7, 109.3, 100.8, 79.0, 69.3, 41.7, 37.2, 32.9, 30.7, 28.3, 28.1. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{18}H_{27}NO_7SNa$: 424.1406. Found: 424.1389.

***tert*-Butyl (2-(6-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-propyl)benzo[*d*][1,3]dioxol-5-yl)ethyl)carbamate (12).** A flame-dried 50 mL Schlenk flask under an argon atmosphere was charged with **11** (0.218 g, 1.25 mmol) dissolved in dry DMF (1 mL). Thereafter, at 0 °C, NaH (45 mg, 1.1 mmol), from a 60% suspension in mineral oil, was added portionwise. After 30 min, **10** (0.100 g, 0.250 mmol), dissolved in a minimum of dry DMF, was slowly added via a syringe during a time period of 60 min. The reaction was stirred overnight while gradually being allowed to reach room temperature.

The resulting reaction mixture was evaporated to dryness and purified directly via flash column chromatography (pentane/EtOAc 7:3), yielding **12** (65.8 mg, 0.137 mmol, 55%) as a clear colorless oil.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 6.65 (s, 1H), 6.62 (s, 1H), 5.87 (s, 2H), 4.71 (brs, 1H), 3.73−3.61 (m, 8H), 3.57 (t, $J$ = 5.2, 2H), 3.45 (t, $J$ = 5.8, 2H), 3.37 (t, $J$ = 5.1, 2H), 3.33−3.14 (m, 2H), 2.71 (t, $J$ = 6.9, 2H), 2.60 (t, $J$ = 7.2, 2H), 1.79 (qui, $J$ = 6.4, 2H), 1.42 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 155.9, 146.2, 145.8, 133.5, 129.8, 109.7, 109.6, 100.8, 79.2, 70.8, 70.8, 70.3, 70.2, 70.1, 50.8, 41.7, 32.9, 31.3, 28.8, 28.5. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{23}H_{36}N_4O_7Na$: 503.2482. Found: 503.2495.

**(9*H*-Fluoren-9-yl)methyl(2-(6-(3-(2-(2-(2-azidoethoxy)-ethoxy)ethoxy)propyl)benzo[*d*][1,3]dioxol-5-yl)ethyl)-carbamate (13).** To a 20 mL flask containing **12** (0.045 g, 0.094 mmol) was added 10 mL of 4 M HCl in dioxane. The resulting solution was left to react overnight and thereafter evaporated to dryness in vacuo. The residue was then dissolved in DCM (1 mL) and DIPEA (33 $\mu$L, 0.19 mmol), and then to the resulting mixture was added 9-fluorenylmethyl *N*-succinimidyl carbonate (0.121 g, 0.377 mmol).

After 5 h, the reaction was quenched with 1 M HCl and evaporated to dryness. The product was purified by flash column chromatography (pentane/EtOAc first 7:3, then changed to 1:1 after isolating Fmoc residues), affording **13** (34 mg, 0.056 mmol, 60% over two steps) as a clear oil.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.77 (d, $J$ = 7.6, 2H), 7.59 (d, $J$ = 7.5, 2H), 7.40 (t, $J$ = 7.7, 2H), 7.32 (t, $J$ = 7.0, 2H), 6.67 (s, 1H), 6.63 (s, 1H), 5.90 (s, 2H), 5.04 (brt, $J$ = 5.4, 1H), 4.42 (d, $J$ = 6.7, 2H), 4.22 (t, $J$ = 6.8, 1H), 3.70−3.59 (m, 8H), 3.60−3.52 (m, 2H), 3.45 (t, $J$ = 6.1, 2H), 3.39−3.29 (m, 4H), 2.75 (t, $J$ = 7.2, 2H), 2.62 (t, $J$ = 7.2, 2H), 1.81 (qui, $J$ = 8.0, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 156.5, 146.3, 145.9, 144.1, 141.4, 133.6, 129.6, 127.8, 127.1, 125.1, 120.1, 109.7, 109.7, 100.9, 70.8, 70.7, 70.2, 70.2, 70.1, 66.5, 50.7, 47.4, 42.2, 32.9, 31.3, 28.8. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{33}H_{38}N_4O_7Na$: 625.2638. Found: 625.2637.

**5-(2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-6-(3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)propyl)benzo[*d*][1,3]-dioxol-2-yl Acetate (14).** Compound **13** (28 mg, 0.048 mmol) was added to a flame-dried 50 mL flask under an argon atmosphere and dissolved in benzene. Thereafter, $Pb(OAc)_4$ (63 mg, 0.14 mmol) was added and the temperature was raised to 80 °C. After 17 h, $Pb(OAc)_4$ (21 mg, 0.048 mmol) was added, and the reaction was stirred for another 15 h.

The reaction was evaporated to dryness in vacuo, diluted with EtOAc, washed with water (×3), and evaporated to dryness in vacuo, affording crude **14**.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.75 (d, $J$ = 7.5, 2H), 7.64 (s, 1H), 7.57 (d, $J$ = 7.4, 2H), 7.38 (t, $J$ = 7.3, 2H), 7.30 (t, $J$ = 7.4, 2H), 6.81 (s, 1H), 6.76 (s, 1H), 5.14 (brt, $J$ = 5.5, 1H), 4.42 (d, $J$ = 6.7, 2H), 4.20 (t, $J$ = 6.2, 1H), 3.68−3.57 (m, 8H), 3.58−3.53 (m, $J$ = 4.7, 2H), 3.44 (t, $J$ = 5.8, 2H), 3.39−3.26 (m, 4H), 2.78 (t, $J$ = 7.2, 2H), 2.65 (t, $J$ = 7.6, 2H), 2.09 (s, 3H), 1.80 (qui, $J$ = 6.8, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 180.4, 169.2, 156.4, 144.0, 143.5, 143.1, 141.4, 134.9, 130.9, 127.7, 127.1, 125.0, 120.0, 112.7, 110.4, 110.2, 70.7, 70.6, 70.1, 70.0, 66.4, 50.7, 47.3, 42.1, 32.9, 31.2, 28.8, 21.1, 18.3. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{35}H_{40}N_4O_9Na$: 683.2693. Found: 683.2703.

**(9*H*-Fluoren-9-yl)methyl 2-(3-(2-(2-(2-azidoethoxy)ethoxy)-ethoxy)propyl)-4,5-dihydroxyphenethylcarbamate (1).** Crude **14** (31 mg, 0.048 mmol) was dissolved in a solution of 10% conc. HCl in MeOH (10 mL). The mixture was reacted overnight, after which the solution was evaporated to dryness in vacuo. Thereafter, the product was purified by flash column chromatography (DCM/$Et_2O$ 7:3 to EtOAc/pentane 7:3), furnishing **1** (8.5 mg, 0.014 mmol, 30% over two steps) as an oil.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.76 (d, $J$ = 7.3, 2H), 7.58 (d, $J$ = 7.2, 2H), 7.40 (t, $J$ = 7.2, 2H), 7.31 (t, $J$ = 7.2, 2H), 6.83 (s, 1H), 6.66 (s, 1H), 6.42 (brs, 1H), 5.67 (brs, 1H), 5.03 (brt, $J$ = 5.9, 1H), 4.41 (d, $J$ = 6.8, 2H), 4.21 (t, $J$ = 7.6, 1H), 3.76−3.59 (m, 8H), 3.60−3.50 (m, 2H), 3.43−3.28 (m, 6H), 2.69 (t, $J$ = 7.2, 2H), 2.61 (t, $J$ = 7.2, 2H), 1.77 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 156.7, 144.1, 142.3, 142.2, 141.5, 132.0, 128.7, 127.8, 127.2, 125.2, 120.1, 117.4, 116.4, 71.0, 70.7, 70.4, 70.1, 70.1, 69.4, 66.7, 50.7, 47.4, 42.2, 32.2, 30.5, 28.1. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{32}H_{38}N_4O_7Na$: 613.2638. Found: 613.2640.

***tert*-Butyl(2-(5-hydroxy-1*H*-indol-3-yl)ethyl)carbamate (16).**[30] Serotonin hydrochloride **15** (1.0 g, 4.7 mmol) was placed in a flame-dried Schlenk flask and dissolved in methanol under an argon atmosphere. Triethylamine (1.51 mL, 10.3 mmol) was added, and the solution was cooled to 0 °C, whereupon $Boc_2O$ (1.54 g, 7.05 mmol) was dissolved in methanol and added dropwise. After 3 h, the resulting mixture was quenched with $H_2O$, transferred to a separation funnel, and extracted three times with DCM. The combined organic phases were dried ($Na_2SO_4$), filtered under suction, and concentrated in vacuo. The product was purified by flash column chromatography (EtOAc/pentane 1:1) and isolated as a yellow solid (1.21 g, 4.38 mmol, 93%).

mp(uncorr.) 114.1−115.0 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.11 (s, 1H), 7.17 (d, $J$ = 8.6, 1H), 6.99 (d, $J$ = 2.0, 1H), 6.92 (s, 1H), 6.80 (dd, $J$ = 2.1, 8.6, 1H), 6.18 (brs, 1H), 4.73 (brs, 1H), 3.47−3.32 (m, 2H), 2.82 (t, $J$ = 6.6, 2H), 1.45 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 156.4, 149.9, 131.7, 128.2, 123.3, 112.4, 112.2, 112.0, 103.4, 79.6, 40.9, 28.6, 26.0. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{15}H_{20}N_2O_3Na$: 299.1372. Found: 299.1369.

***tert*-Butyl(2-(5-(allyloxy)-1*H*-indol-3-yl)ethyl)carbamate (17).** Boc-protected serotonin **16** (3.33 g, 12.1 mmol) was placed in a dry Schlenk flask under an argon atmosphere and dissolved in acetone. Thereafter, $Cs_2CO_3$ (9.82 g, 30.1 mmol) was added, and after 10 min of stirring, allyl iodide (2.75 mL, 30.1 mmol) was added. The resulting yellow solution was stirred overnight, after which the reaction was filtered under suction and evaporated to dryness in vacuo. The compound was purified by flash column chromatography (EtOAc/pentane 3:7), affording **17** as a yellow solid (2.82 g, 8.91 mmol, 74%).

mp (uncorr.) 88.0−88.7 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.05 (s, 1H), 7.25 (d, $J$ = 8.8, 1H), 7.06 (d, $J$ = 1.8, 1H), 7.00 (s, 1H), 6.90 (dd, $J$ = 2.4, 8.8, 1H), 6.33−5.96 (m, 1H), 5.45 (dq, $J$ = 1.6, 17.3, 1H), 5.29 (dq, $J$ = 1.4, 10.5, 1H), 4.65 (brs, 1H), 4.59 (dt, $J$ = 1.4, 5.3, 2H), 3.63−3.30 (m, 2H), 2.91 (t, $J$ = 6.5, 2H), 1.44 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 156.2, 152.7, 133.9, 131.8, 127.6, 123.1, 117.3, 112.6, 112.3, 112.0, 102.2, 79.2, 69.9, 40.9, 28.4, 25.7. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{18}H_{24}N_2O_3Na$: 339.1685. Found: 339.1680.

***tert*-Butyl(2-(4-allyl-5-hydroxy-1*H*-indol-3-yl)ethyl)-carbamate (18).** Compound **17** (3.33 g, 10.5 mmol) was dissolved in *o*-xylene (100 mL) under an argon atmosphere and refluxed overnight. The solvent was evaporated in vacuo, and thereafter, the product was purified by flash column chromatography (EtOAc/pentane 4:6), affording **18** as a yellow solid (2.12 g, 6.72 mmol, 64%).


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mp (uncorr.) 120.9−121.4 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.03 (s, 1H), 7.12 (d, $J$ = 8.6, 1H), 6.96 (s, 1H), 6.79 (d, $J$ = 8.6, 1H), 6.19−5.98 (m, 1H), 5.11 (d, $J$ = 10.1, 1H), 5.01 (d, $J$ = 17.3, 1H), 4.67 (brs, 1H), 3.78 (d, $J$ = 5.3, 2H), 3.58−3.26 (m, 2H), 3.02 (t, $J$ = 6.5, 2H), 1.47 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 156.2, 147.9, 136.9, 132.6, 126.3, 123.7, 115.8, 113.1, 112.9, 110.2, 79.4, 41.5, 30.5, 28.6, 27.7. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{18}H_{24}N_2O_3Na$: 339.1685. Found: 339.1662.

***tert*-Butyl(2-(4-allyl-5-((tetrahydro-2*H*-pyran-2-yl)oxy)-1*H*-indol-3-yl)ethyl)carbamate (19).** Compound **18** (0.412 g, 1.30 mmol) was dissolved in dry DCM (65 mL), after which pyridinium *p*-toluenesulfonate (49.2 mg, 0.196 mmol) and dihydropyran (0.13 mL, 1.4 mmol) were added. After 3 h, the reaction was quenched by adding water, extracted three times with EtOAc, dried ($Na_2SO_4$), and evaporated to dryness in vacuo. The product was purified via flash column chromatography (EtOAc/pentane first 2:8, then 3:7), resulting in **19** (0.320 g, 0.800 mmol, 61%) as a light-sensitive oil.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.19 (s, 1H), 7.15 (d, $J$ = 8.8, 1H), 7.11 (d, $J$ = 8.8, 1H), 6.94 (s, 1H), 6.08 (m, 1H), 5.26 (t, $J$ = 3.3, 1H), 4.99 (dq, $J$ = 1.6, 10.1, 1H), 4.88 (dq, $J$ = 1.8, 17.1, 1H), 4.69 (brs, 1H), 4.07−3.95 (m, 1H), 3.91−3.69 (m, 2H), 3.67−3.54 (m, 1H), 3.51−3.37 (m, 2H), 3.04 (t, $J$ = 6.7, 2H), 1.95−1.82 (m, 2H), 1.74−1.54 (m, 4H), 1.45 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 156.1, 149.1, 138.2, 133.3, 126.2, 123.6, 120.6, 114.7, 113.3, 112.9, 109.7, 98.8, 79.3, 62.4, 41.5, 31.0, 30.5, 28.6, 27.5, 25.5, 19.4. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{23}H_{32}N_2O_4Na$: 423.2260. Found: 423.2252.

***tert*-Butyl-4-allyl-3-(2-((*tert*-butoxycarbonyl)amino)ethyl)-5-((tetrahydro-2*H*-pyran-2-yl)oxy)-1*H*-indole-1-carboxylate (20).** To a dry flask were added $Boc_2O$ (0.104 g, 0.478 mmol), DMAP (83 mg, 0.68 mmol), and $Et_3N$ (0.19 mL, 1.3 mmol), and thereafter, the compounds were dissolved in dry THF (8 mL). The resulting solution was cooled to 0 °C, after which **19** (0.182 g, 0.455 mmol) was added dropwise as a suspension in dry THF (8 mL). After 2 h, the reaction was extrachted with DCM (×3), dried ($Na_2SO_4$), filtered under suction, and evaporated in vacuo.

The product was purified by flash column chromatography (EtOAc/pentane 1:9), affording **20** as an oil (0.133 g, 0.266 mmol, 58%).

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.00 (d, $J$ = 9.0, 1H), 7.35 (s, 1H), 7.18 (d, $J$ = 9.1, 1H), 6.04 (ddt, $J$ = 5.3, 10.3, 15.6, 1H), 5.33 (t, $J$ = 3.1, 1H), 4.98 (dd, $J$ = 1.6, 10.2, 1H), 4.80 (dd, $J$ = 1.7, 17.2, 1H), 4.74 (bs, 1H), 4.00−3.87 (m, 1H), 3.86−3.65 (m, 2H), 3.64−3.52 (m, 1H), 3.51−3.36 (m, 2H), 2.98 (t, $J$ = 6.8, 2H), 2.03−1.93 (m, 1H), 1.92−1.83 (m, 2H), 1.72−1.55 (m, 12H), 1.44 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 155.9, 151.0, 149.5, 137.6, 131.6, 129.4, 124.3, 120.7, 118.0, 114.8, 113.7, 113.0, 97.6, 83.2, 79.1, 62.0, 40.6, 30.7, 29.8, 28.4, 28.2, 27.5, 25.3, 19.0. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{28}H_{40}N_2O_6Na$: 523.2784. Found: 523.2787.

***tert*-Butyl-3-(2-((*tert*-butoxycarbonyl)amino)ethyl)-4-(3-hydroxypropyl)-5-((tetrahydro-2*H*-pyran-2-yl)oxy)-1*H*-indole-1-carboxylate (21).** To a 0 °C solution of **20** (0.133 g, 0.266 mmol) in dry THF was added $BH_3$ (0.56 mL, 0.56 mmol), from a 1 M solution in THF. After 3 h, NaOH (0.53 mL, 0.53 mmol) from a 1 M aq. solution and $H_2O_2$ (0.12 mL, 1.2 mmol) from a 35% aq. solution were added, and the resulting mixture was left to react for 3 h. Thereafter, the reaction was diluted with water and extracted with $Et_2O$ (×4) and DCM (×3). The combined organic phases were evaporated in vacuo, and thereafter, the product was purified by flash column chromatography (EtOAc/pentane 4:6), obtaining the product as a yellow oil (0.097 g, 0.19 mmol, 70%).

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.95 (d, $J$ = 9.0, 1H), 7.36 (s, 1H), 7.15 (d, $J$ = 9.1, 1H), 5.35 (t, $J$ = 3.2, 1H), 4.99 (t, $J$ = 5.4, 1H), 3.98−3.87 (m, 1H), 3.79−3.50 (m, 3H), 3.46−3.33 (m, 2H), 3.28−2.88 (m, 4H), 2.06−1.76 (m, 5H), 1.75−1.57 (m, 12H), 1.44 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 156.4, 150.9, 149.6, 131.9, 129.0, 124.8, 123.8, 118.1, 113.4, 113.1, 98.0, 83.4, 79.6, 62.4, 41.7, 34.5, 30.9, 28.5, 28.3, 28.1, 27.9, 25.3, 22.6, 19.3. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{28}H_{42}N_2O_7Na$: 541.2890. Found: 541.2891.

***tert*-Butyl-3-(2-((*tert*-butoxycarbonyl)amino)ethyl)-4-(3-((methylsulfonyl)oxy)propyl)-5-((tetrahydro-2*H*-pyran-2-yl)oxy)-1*H*-indole-1-carboxylate (22).** To a 0 °C solution of **21** (0.144 g, 0.278 mmol) in dry $Et_2O$ (30 mL) were added $Et_3N$ (77 $\mu$L, 0.56 mmol) and MsCl (32 $\mu$L, 0.42 mmol). After 2 h, an additional amount of $Et_3N$ (77 $\mu$L, 0.56 mmol) and MsCl (32 $\mu$L, 0.42 mmol) was added, and the reaction was left to react overnight. Thereafter, the reaction was diluted with water and extracted with EtOAc (×4). The combined organic phases were evaporated to dryness, and then the product was purified by flash column chromatography (EtOAc/pentane 3:7), yielding **22** as an oil (152 mg, 0.254 mmol, 92%).

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.99 (d, $J$ = 9.2, 1H), 7.38 (s, 1H), 7.16 (d, $J$ = 9.1, 1H), 5.37 (t, $J$ = 3.2, 1H), 4.94 (bs, 1H), 4.34 (t, $J$ = 6.1, 2H), 3.96−3.86 (m, 1H), 3.66−3.58 (m, 1H), 3.50−3.37 (m, 2H), 3.25−3.03 (m, 2H), 3.03−2.93 (m, 5H), 2.16−1.80 (m, 5H), 1.77−1.65 (m, 2H), 1.65−1.60 (m, 10H), 1.45 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 156.1, 151.0, 149.5, 131.8, 129.0, 124.9, 122.0, 117.7, 113.9, 112.7, 97.8, 83.5, 79.3, 70.4, 62.5, 41.1, 37.4, 31.0, 30.7, 28.5, 28.3, 28.1, 25.4, 22.6, 19.4. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{29}H_{44}N_2O_9SNa$: 619.2665. Found: 619.2679.

***tert*-Butyl(2-(4-(3-((2-(2-(2-azidoethoxy)ethoxy)ethyl)thio)-propyl)-5-((tetrahydro-2*H*-pyran-2-yl)oxy)-1*H*-indol-3-yl)-ethyl)carbamate (24a) and *tert*-Butyl-4-(3-((2-(2-(2-azidoethoxy)ethoxy)ethyl)thio)propyl)-3-(2-((*tert*-butoxycarbonyl)amino)ethyl)-5-((tetrahydro-2*H*-pyran-2-yl)-oxy)-1*H*-indole-1-carboxylate (24b).** To a 0 °C solution of **23** (98.4 mg, 0.514 mmol) in dry DMF (0.3 mL) under an argon atmosphere was added NaH (13.2 mg, 0.329 mmol) from a 60% suspension in mineral oil. After 40 min, **22** (61.4 mg, 0.103 mmol), dissolved in a minimum amount of dry DMF, was added, and the reaction was left to react overnight. Thereafter, the reaction was quenched with water, extracted with EtOAc (×4), and evaporated to dryness in vacuo.

The resulting residue was purified via flash column chromatography (gradient: first isolating **24a** via pentane/EtOAc (7:3) and **24b** in pentane/EtOAc 6:4; any linker residues removed by second column in acetone/pentane 2:8), resulting in **24a** (22.8 mg, 0.0385 mmol, 37%) and **24b** (10.2 mg, 0.0147 mmol, 14%) as an oil, obtaining a combined yield of 51%.

***24a*:** $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.08 (brs, 1H), 7.13 (d, $J$ = 8.8, 1H), 7.09 (d, $J$ = 8.8, 1H), 6.97 (s, 1H), 5.28 (t, $J$ = 3.3, 1H), 4.80 (brs, 1H), 4.04−3.92 (m, 1H), 3.70−3.56 (m, 9H), 3.49−3.41 (m, 2H), 3.41−3.30 (m, 2H), 3.20−2.96 (m, 4H), 2.78−2.64 (m, 4H), 2.11−1.83 (m, 5H), 1.73−1.57 (m, 3H), 1.45 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 156.1, 148.9, 133.2, 125.8, 123.7, 122.8, 113.2, 112.4, 109.4, 98.5, 79.2, 71.1, 70.8, 70.4, 70.2, 62.5, 50.8, 41.7, 32.9, 31.6, 31.5, 31.2, 28.6, 27.9, 26.1, 25.5, 19.5. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{29}H_{45}N_5O_6SNa$: 614.2988. Found: 614.2988.

***24b*:** $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.05−7.86 (m, 1H), 7.36 (s, 1H), 7.16 (d, $J$ = 9.1, 1H), 5.37 (t, $J$ = 2.9, 1H), 4.77 (brs, 1H), 3.99−3.84 (m, 1H), 3.74−3.55 (m, 9H), 3.54−3.30 (m, 4H), 3.17−2.91 (m, 4H), 2.73 (t, $J$ = 7.0, 2H), 2.68 (t, $J$ = 7.1, 2H), 2.14−1.77 (m, 5H), 1.75−1.60 (m, 12H), 1.45 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 155.9, 150.8, 149.5, 131.6, 128.9, 124.6, 123.1, 117.7, 113.4, 112.6, 97.5, 83.3, 79.2, 71.0, 70.6, 70.3, 70.0, 62.2, 50.7, 40.9, 38.4, 32.7, 31.6, 31.3, 30.9, 28.4, 28.2, 25.5, 25.3, 19.2. HRMS (ES) $m/z$ [M + H]$^+$ calcd for $C_{34}H_{54}N_5O_8S$: 692.3693. Found: 692.3694.

**(9*H*-Fluoren-9-yl)methyl (2-(4-(3-((2-(2-(2-azidoethoxy)-ethoxy)ethyl)thio)propyl)-5-hydroxy-1*H*-indol-3-yl)ethyl)-carbamate (2).** To a solution of **24a** (9.7 mg, 0.016 mmol) and **24b** (6.6 mg, 0.0095 mmol) in MeOH (10 mL) was added HCl (9.5 mL) from a 4 M HCl solution in dioxane, and the mixture was reacted overnight. Thereafter, the mixture was evaporated to dryness in vacuo. The resulting residue was redissolved in toluene and evaporated to dryness (repeated three times). Thereafter, the residue was dissolved in dry DCM (6 mL), and DIPEA (9 $\mu$L, 0.026 mmol) was added, followed by addition of 9-fluorenylmethyl *N*-succinimidyl carbonate (16 mg, 0.047 mmol), which was added portionwise. After 3 h, the reaction was quenched with 1 M HCl, evaporated to dryness in vacuo, and purified via flash column chromatography (EtOAc/pentane 4:6), affording **2** (5.5 mg, 0.0087 mmol, 34% over two steps).

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.91 (s, 1H), 7.77 (d, $J$ = 7.6, 2H), 7.59 (d, $J$ = 7.2, 2H), 7.40 (t, $J$ = 7.4, 2H), 7.31 (t, $J$ = 7.6, 2H), 7.10


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(d, $J$ = 8.6, 1H), 6.94 (s, 1H), 6.77 (d, $J$ = 8.6, 1H), 5.02 (t, $J$ = 7.5, 1H), 4.43 (d, $J$ = 6.8, 2H), 4.22 (t, $J$ = 6.6, 1H), 3.68−3.57 (m, 8H), 3.57−3.47 (m, 3H), 3.40−3.31 (m, 2H), 3.14−2.99 (m, 4H), 2.74 (t, $J$ = 6.7, 2H), 2.69 (t, $J$ = 6.7, 2H), 2.03−1.91 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 156.6, 147.7, 144.1, 141.5, 132.5, 127.8, 127.2, 125.9, 125.2, 123.7, 120.1, 118.3, 112.9, 112.8, 109.8, 71.1, 70.8, 70.5, 70.1, 66.6, 50.8, 47.5, 42.2, 32.3, 31.6, 30.5, 27.8, 24.8. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_{34}H_{39}N_5O_5SNa$: 652.2570. Found: 652.2572.

**2,5-Dioxopyrrolidin-1-yl pent-4-ynoate (26).**[26] To a solution of 4-pentynoic acid (0.100 g, 1.02 mmol) in DCM (5 mL) were added *N*-hydroxysuccinimide (0.134 g, 1.17 mmol) and EDC (0.391 g, 2.04 mmol). After 2 h, the reaction was transferred to a separating funnel and washed with a 2.5% aq. solution of $NaHSO_4$ (×3). The organic phase was subsequently dried ($Na_2SO_4$), filtered under suction, and evaporated in vacuo to yield **26** as a white solid (0.191 g, 0.980 mmol, 96%).

mp(uncorr.) 79.9−80.5 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 2.92−2.80 (m, 6H), 2.62 (dt, $J$ = 2.7, 7.1, 2H), 2.05 (t, $J$ = 2.7, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 169.1, 167.1, 81.0, 70.1, 30.2, 25.5, 14.0. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_9H_9NO_4Na$: 218.0429. Found: 218.0429.

**2-(2-(2-Azidoethoxy)ethoxy)ethyl Methanesulfonate (30).** To a 0 °C solution of **11** (0.500 g, 2.85 mmol) in dry $Et_2O$ (10 mL) were added $Et_3N$ (1.2 mL, 8.56 mmol) and, thereafter, MsCl (0.33 mL, 4.28 mmol). The resulting white slurry was quenched with water after 24 h and extracted with $Et_2O$ (×3). The combined organic phases were dried ($MgSO_4$), filtered under suction, and purified via flash column chromatography (pentane/EtOAc 1:1), yielding **30** (0.600 g, 2.37 mmol, 83%) as a clear oil.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 4.35−4.21 (m, 2H), 3.73−3.65 (m, 2H), 3.63−3.53 (m, 6H), 3.31 (t, $J$ = 5.2, 2H), 2.99 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 70.4, 70.4, 69.8, 69.3, 68.9, 50.5, 37.4. HRMS (ES) $m/z$ [M + H]$^+$ calcd for $C_7H_{16}N_3O_5S$: 254.0811. Found: 254.0811.

***S*-(2-(2-(2-Azidoethoxy)ethoxy)ethyl) Ethanethioate (31).** To a solution of **30** (0.348 g, 1.37 mmol) in abs. EtOH (225 mL) was added potassium thioacetate (0.863 g, 7.56 mmol). The resulting mixture was heated to reflux overnight. The orange mixture was then cooled to rt, quenched with water, and extracted with DCM (×3). The combined organic phases were dried ($MgSO_4$), filtered under suction, and purified via flash column chromatography (EtOAc/pentane 2:8), yielding **31** (0.288 g, 1.23 mmol, 90%) as a yellow oil.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 3.69−3.42 (m, 9H), 3.33 (t, $J$ = 5.1, 2H), 3.03 (t, $J$ = 6.4, 2H), 2.25 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 195.4, 70.5, 70.3, 70.0, 69.7, 50.6, 30.5, 28.7. HRMS (ES) $m/z$ [M + H]$^+$ calcd for $C_8H_{16}N_3O_3S$: 234.0912. Found: 234.0917.

**2-(2-(2-Azidoethoxy)ethoxy)ethanethiol (23).** Sodium (0.05 g, 2.17 mmol) was added to MeOH (5 mL). After the generation of NaOMe was complete, the mixture was added to a solution of **31** (0.253 g, 1.09 mmol) in MeOH (15 mL). The resulting mixture was heated to 50 °C and reacted for 2 days (monitored by TLC). Thereafter, the reaction was acidified by the addition of 0.1 M HCl, brine was added, and the mixture was extracted with DCM (×3). The combined organic phases were dried ($MgSO_4$), filtered under suction, and purified via flash column chromatography (EtOAc/pentane 3:7), yielding **23** (0.175 mg, 0.915 mmol, 84%) as an oil.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 3.74 (t, $J$ = 6.7, 2H), 3.71−3.58 (m, 6H), 3.39 (t, $J$ = 5.0, 2H), 2.89 (t, $J$ = 6.7, 2H), 2.17 (s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 70.8, 70.5, 70.2, 69.8, 50.8, 38.5. HRMS (ES) $m/z$ [M + Na]$^+$ calcd for $C_6H_{14}N_3O_2SNa$: 214.0626. Found: 214.0627.

**Alkyne Functionalized Dynabeads M-270 Amine (27).** The supernatant of an aqueous solution of Dynabeads (M-270 amine, Invitrogen, 1.5 mL, 2 × 10$^9$ beads/mL) was removed by magnetically immobilizing the beads. Thereafter, the beads were washed with DMF and added to a solution of **26** (50 mM), DIPEA (12.5 $\mu$L), and water (112.5 $\mu$L) in DMF (375 $\mu$L). The beads were left to react for 30 min. The coupling step was performed a total of three times. Thereafter, the beads were washed with DMF (3 × 500 $\mu$L) and subsequently acetylated by suspending the beads in acetic anhydride (20 mM) and DIPEA (50 $\mu$L) in DMF (950 $\mu$L). The acetylation reaction was performed for 30 min, and the beads were washed with DMF (5 × 500 $\mu$L). Finally, the beads were resuspended in DMF (1 mL) and kept at 5 °C until further use.

**Dopamine Derivative 1 Functionalized Dynabeads M-270 Amine (28).** To an Eppendorff tube was transferred 0.250 mL from a stock solution of **27** in DMF (1 mL). The supernatant was removed, whereafter a solution of **1** (18 mM), $CuSO_4$ (200 $\mu$M), THPTA[31] (1.4 mM), and NaAsc (2 mM) in a water/DMF (1:4) mixture (500 $\mu$L) was added. The reaction was shaken for 1 h at room temperature, and then the supernatant was removed. The remaining beads were washed with water/DMF (1:1) (2 × 500 $\mu$L), water (3 × 500 $\mu$L), water/DMF (1:1) (1 × 500 $\mu$L), and DMF (3 × 500 $\mu$L). Thereafter, 50% piperidine in DMF (500 $\mu$L) was added, and the beads were shaken for 90 min. The supernatant was removed, and the beads were again suspended in 50% piperidine in DMF. The supernatant was then removed and combined with the first deprotection mixture. The concentration of 9-methylidenefluorene was measured by a determined max UV abs. between 301 and 298 nm (7800 M$^{-1}$ cm$^{-1}$),[32] furnishing an estimated amount of 30 nmol. The beads were stored in a freezer suspended in a 1 mM ascorbic acid, 150 mM NaCl, 80 mM HEPES buffer (pH = 7.4) in order to avoid oxidation of the neurotransmitter analogues.

**Serotonin Derivative 2 Functionalized Dynabeads M-270 Amine (29).** To an Eppendorff tube was transferred 1 mL from a stock solution of **27** in DMF (1 mL). The supernatant was removed, whereafter a solution of **2** (35 mM), $CuSO_4$ (200 $\mu$M), THPTA[31] (1.4 mM), and NaAsc (2 mM) in a water/DMF (1:4) mixture (500 $\mu$L) was added. The reaction was shaken for 1 h at room temperature, and then the supernatant was removed. The remaining beads were washed with water/DMF (1:1) (2 × 500 $\mu$L), water (3 × 500 $\mu$L), water/DMF (1:1) (1 × 500 $\mu$L), and DMF (3 × 500 $\mu$L). Thereafter, 50% piperidine in DMF (500 $\mu$L) was added, and the beads were shaken for 90 min. The supernatant was removed, and the beads were again suspended in 50% piperidine in DMF. Thereafter, the supernatant was removed and combined with the first deprotection mixture. The concentration of 9-methylidenefluorene was measured by a determined max UV abs. between 301 and 298 nm (7800 M$^{-1}$ cm$^{-1}$),[32] furnishing an estimated amount of 98 nmol. The beads were stored in a freezer suspended in a 1 mM ascorbic acid, 150 mM NaCl, 80 mM HEPES buffer (pH = 7.4) in order to avoid oxidation of the neurotransmitter analogues.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

$^1$H NMR and $^{13}$C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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