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2,4-Bis(hydroxymethyl)aniline as a Building Block for Oligomers with Self-Eliminating and Multiple Release Properties

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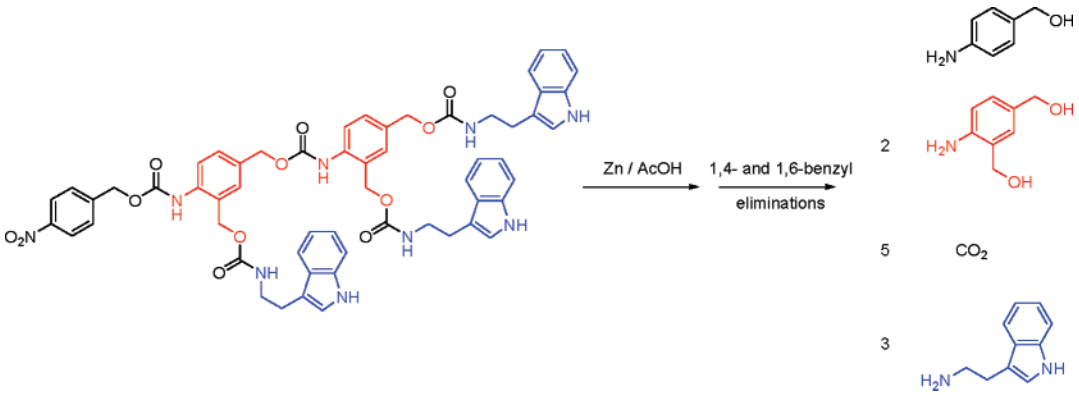

Linear self-eliminating (LSE) systems are oligomers of branched self-eliminating linkers that disassemble upon a single triggering event under complete degradation of the linear backbone, accompanied by the release of side-chain bound effector molecules. Enabling a controlled and almost simultaneous release of different effectors (drugs) in defined ratios, LSE systems may gain importance for the development of novel combination therapeutics. On the basis of the well-known self-eliminating *p*-aminobenzyloxycarbonyl (PABC) linker, 2,4-bis(hydroxymethyl)aniline was considered a suitable branched linker for building LSE systems that degrade by 1,6- and 1,4-benzyl elimination reactions. A first LSE model system based on this linker was prepared in a simple procedure and was shown to release its effector payload efficiently after activation. In addition, elimination model compounds were synthesized to study the release behavior of LSE systems based on 2,4-bis(hydroxymethyl)aniline. It was found that chain degrading 1,6-benzyl elimination occurs much faster than the effector releasing 1,4-elimination.

Introduction

For developing more sophisticated prodrug technologies for cancer chemotherapy, the interest in chemical architectures that enable a site-specific release of carrier-bound drugs has grown rapidly over the last years.[1,2] Self-immolative dendrimers are recent examples of complex molecular structures designed for a controlled and multiple release of small molecules.[3,4] Based on self-eliminating linkers as branching units, self-immolative dendrimers can be terminally loaded with various effector/reporter molecules. Activation at the focal point initiates a cascade of elimination reactions which eventually lead to a breakdown of the whole dendritic scaffold with a concomitant release of the molecular payload. This simultaneous multiple release of effector molecules upon a single activation step makes these compounds attractive for a use as "intelligent" carriers for drugs or imaging agents. Nevertheless, there are some present drawbacks of this concept: The main problem is that only a restricted number of drug molecules fit into the limited space of the outer shell of the dendrimer. G3 dendrons with eight small dye molecules[5,6] and a G2 dendron with four molecules of the bulky drug paclitaxel[7] are the largest self-eliminating dendrimer conjugates that could be synthesized up to now. Furthermore,

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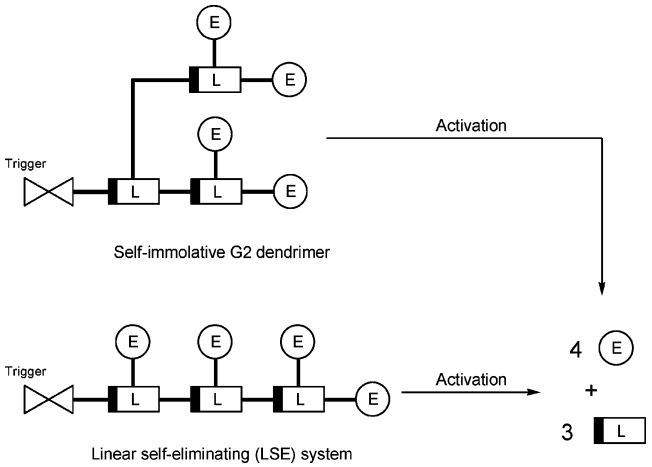
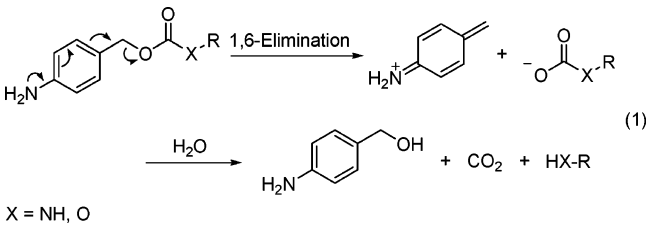


FIGURE 1. Examples for a dendritic and a linear implementation of a self-eliminating structure comprised of three linker units (L) that disassemble upon activation to release four effector molecules (E).

dendritic structures are not suitable for conveniently combining different drugs. For instance, 13 steps were necessary to synthesize a G1 dendrimer that was loaded with one molecule each of the anticancer drugs camptothecin, etoposide, and doxorubicin.[8]

For designing novel combination therapeutics with variable drug ratios,[9] nondendritic carrier topologies, i.e., linear self-eliminating (LSE) systems, are likely to offer more potential. Such linear systems are based on branched self-eliminating linkers as monomer units—the same as for self-immolative dendrimers (Figure 1). For *n* effector/reporter molecules, *n* − 1 linker units must be incorporated. Chemical or enzymatic activation of the trigger causes the molecule to disassemble in two directions: the bonds between two linkers that form the linear backbone as well as the bonds between the linkers and the effector molecules are cleaved by elimination reactions. For drug delivery applications, this disassembly should occur sufficiently fast in aqueous media to ensure high local drug levels.

Very efficient self-eliminating structures are based on *p*-aminobenzyloxycarbonyl (PABC) linkers first introduced by Katzenellenbogen.[10] With the amino group acylated or otherwise protected, the system is considerably stable under physiologic conditions whereas the linker with a free amino group undergoes a rapid 1,6-elimination of unstable carbamic or carbonic acids which in turn decarboxylate to the respective amines or alcohols.



(1)

The intermediately formed iminoquinone methide is trapped by nucleophiles (water) and thereby regenerates the aminobenzyl system. Since respective *o*-aminobenzyl derivatives show an analogous reaction (1,4-elimination),[11] 2,4-bis(hydroxymethyl)-aniline (**1**) was chosen as an appropriate branched double release linker for our approach. Scheme 1 shows one of the two possible disassembly mechanisms for LSE systems based on **1**: 1,6-elimination leads to the breakdown of the linear chain followed by release of the effectors through 1,4-elimination (in theory, these reactions could also take place in reversed order). Apparently, the rates for both benzyl elimination reactions as well as for the regeneration of the aminobenzyl system from the quinoidal intermediates are crucial for the double-release efficiency.

In this work, we describe the synthesis of monoprotected 2,4-bis(hydroxymethyl)aniline and its use as a self-eliminating double release linker and building block for LSE systems. Model compounds with tryptamine were prepared to study the elimination properties. Furthermore, by appending two linker units, a first linear self-eliminating system was constructed. Elimination kinetics of all model compounds were determined by using HPLC.

Results

As a straightforward approach for the construction of effector/reporter-loaded, carbamate-linked oligomers of **1**, we made use of a simple two-step procedure: (1) conversion of the 4-hydroxybenzyl group into an activated 4-nitrophenyl (Np) carbonate and (2) appending another linker through its amino group by forming a carbamate bond. Unfortunately, it is not possible to employ linker-effector derivatives as building blocks in this strategy since effective linkers with an unprotected amino group will immediately undergo elimination of the effector. Hence, we planned to use monoprotected **1** for building up the linear backbone followed by removal of the protective groups, activation with 4-nitrophenyl chloroformate, and coupling with the amine-containing reporter molecule. Monoprotected 2,4-bis-(hydroxymethyl)aniline was readily prepared in a 5-step procedure starting with ethyl 3-methyl-4-nitrobenzoate (Scheme 2). After radical bromination, conversion to the benzyl alcohol using silver sulfate, TBS protection, and a successive reduction of both nitro and ester group, linker **6** was obtained in an acceptable overall yield.

To evaluate the potential of **1** as a building block for LSE systems, we were interested in answering the following questions: (1) Do single 1,4- and 1,6-benzylelimination reactions proceed with comparable reaction rates? (2) Do they also occur when combined in a double elimination system? (3) Does chain degradation in a LSE system occur with the same rate as the release of the reporter molecules?

To clarify these issues we developed four different model compounds. First, we synthesized the single 1,4- and 1,6-benzylelimination model systems **8a** and **8b** (Scheme 3) that are tryptamine-carbamoyl derivatives of 4- and 2-nitrobenzyl alcohol, respectively. Since reduction of aromatic nitro groups is a facile and fast reaction, **8a** and **8b** are suitable precursors for the respective aminobenzyl compounds. Tryptamine serves as a model for an amino-functionalized effector/drug and can be readily quantified using HPLC. To study the double release behavior of bis-carbamoyl derivatives of **1**, model compound **12** bearing two molecules of tryptamine was synthesized from 4-nitrobenzyl alcohol and **6** in 5 steps (Scheme 3). Furthermore,

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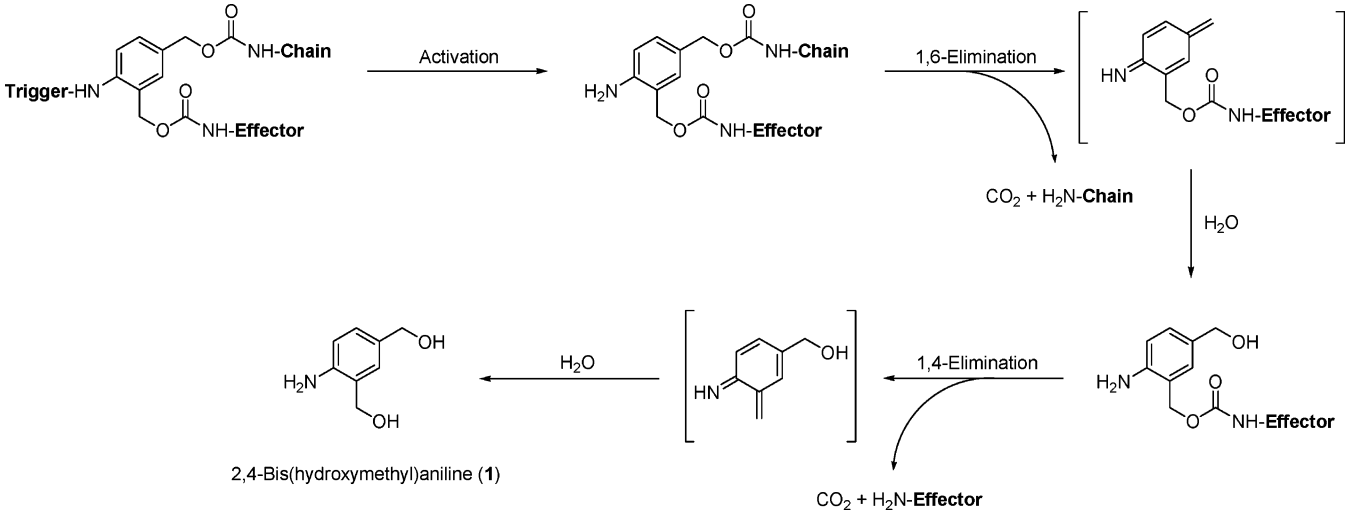
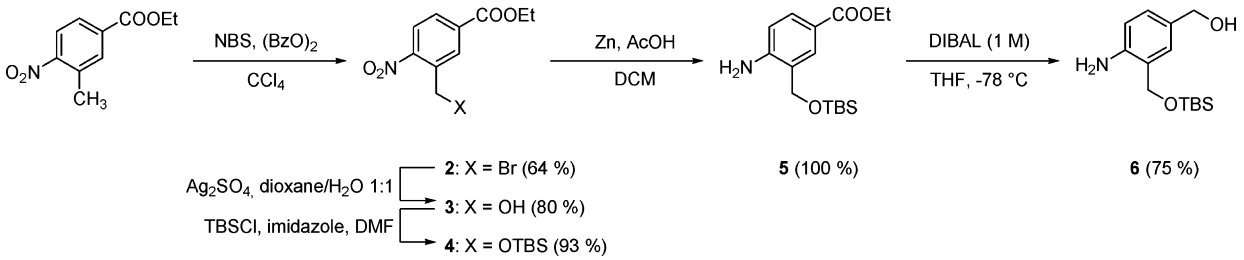
SCHEME 1. One of Two Possible Disassembly Pathways of 2,4-Bis(hydroxymethyl)aniline-Based Linear Self-Eliminating (LSE) Systems in Aqueous Media



SCHEME 2. Synthesis of 6



by applying the above-mentioned strategy of two alternating activation and coupling steps, followed by removal of all protective groups, activation with 4-nitrophenyl chloroformate, and reaction with tryptamine, compound **17** was obtained as a first simple LSE system that enables the release of three effector molecules (Scheme 3). In analogy to **8a** and **8b**, self-disassembly of **12** and **17** was triggered by reduction of the 4-nitrobenzyloxycarbonyl group. The formed self-eliminating PABC linker then initiates the cascade of elimination reactions.

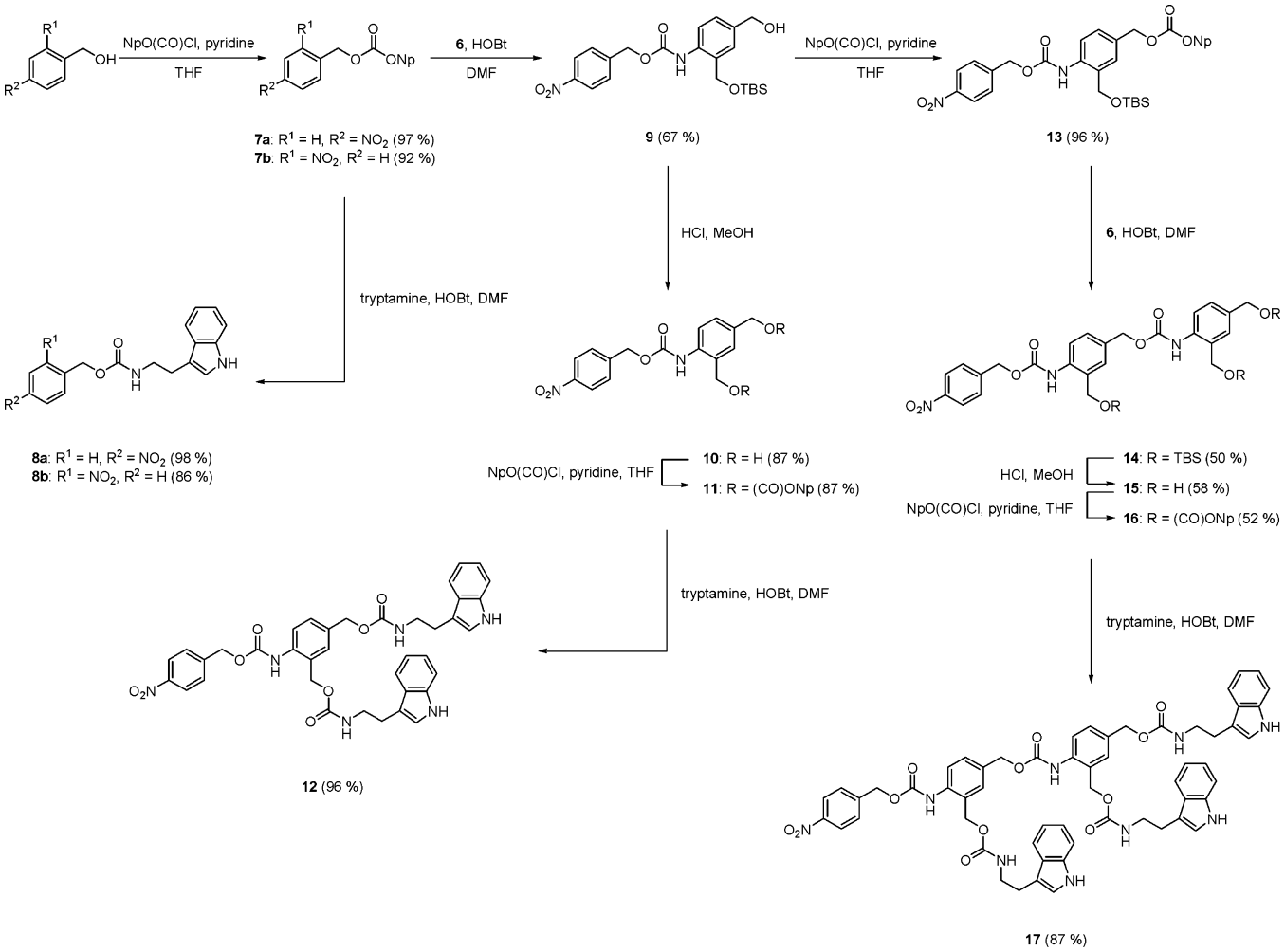
Reduction of the nitro groups was accomplished by treating the model compounds with an excess of zinc powder and 5% AcOH in MeCN. Under these conditions, a reaction time of 1 min proved to be sufficient for complete conversion of the starting material as shown by HPLC. Aqueous dilutions of the samples (containing ~0.3% AcOH, pH 3) were stored at 37 °C and analyzed at appropriate intervals. For determination of the disassembly kinetics the formed tryptamine was quantified. Divided by the tryptamine concentrations c_∞ for complete conversion (determined after adequately long reaction times), relative tryptamine concentrations c/c_∞ were obtained. Figure 2 shows the time-dependent release of tryptamine from **8a**, **8b**, **12**, and **17**. To verify that the observed release of tryptamine is due to benzyl elimination reactions and does not result from an unspecific hydrolysis of the carbamates, analogous experiments were carried out without the addition of zinc powder. For all model compounds no substantial release of tryptamine could be detected after one week storage at pH 3 and 37 °C.

Discussion

One major aim of our work was to develop a facile synthetic approach to the first linear self-eliminating system. In our strategy we employed **6** as a protected building block that was activated, dimerized, and loaded with tryptamine as a reporter to afford **17** as a first model LSE system. Compound **17** was shown to disassemble upon reduction of the nitro group as indicated by the formation of tryptamine with >50% of the reporter being released after 4 h (Figure 2). To gain deeper insights into the elimination mechanism of reduced **17**, model compounds **8a**, **8b**, and **12** were synthesized that release tryptamine in a single 1,6-benzyl elimination, a single 1,4-benzyl elimination, and a combined 1,6- and 1,4-benzyl elimination, respectively. After reduction of the nitro groups, all model compounds underwent self-elimination (Figure 2) albeit the release of tryptamine from **8a** (1,6-benzyl elimination) occurred much more rapidly than from the other model compounds with at least one 1,4-benzyl elimination step being involved. In a logarithmic plot (Figure 3) the expected first-order elimination kinetics of the reduced forms of **8a** and **8b** was found in a linear correlation between $\ln(1 - c/c_\infty)$ and t. The calculated half-lives (37 °C, pH 3) are ~4 min for the 1,6-benzyl elimination (**8a**) and ~120 min for the 1,4-elimination reaction (**8b**), which is a remarkable difference of at least 1 order of magnitude.

When analyzing the tryptamine release behavior of the double release compound (reduced **12**), three stages can be clearly distinguished (Figures 2 and 3): a rapid release within the first 20 min followed by a phase of relative stagnation (20−45 min) and a constant, but slower release (45−150 min). Comparing the slope of the logarithmic plot of reduced **12** with the linearized graphs of the single benzyl eliminations (Figure 3), it can be assumed that the first stage is the 1,6-benzyl elimination of the first tryptamine molecule and the last stage the respective 1,4-elimination of the second reporter molecule. The observed

SCHEME 3. Synthesis of Model Compounds



stagnation phase could be explained by the required regeneration of the aminobenzyl system from the chinoidal intermediate. It is therefore most likely that disassembly of the LSE system (**17**) mainly proceeds according to the mechanism depicted in Scheme 1. Due to the more complex elimination mechanism of reduced **17**, however, an equally characteristic release pattern as observed for reduced **12** could not be found.

Liberating >50% of the reporter molecule within 4 h, the release efficiency of **17** proved to be satisfactory for biomedical applications, even under acidic conditions (pH 3). Since it can be assumed that only the aniline form of the linkers will undergo elimination, a strong influence of the pH on the elimination rate should be expected. Assuming further that the pK_a of protonated **1** is in the same range as for anilinium (pK_a = 4.6), a high percentage of the amino groups are protonated at the reaction conditions used (0.3% AcOH, pH 3) thus making the reaction sufficiently slow to be analyzed by HPLC. For the elimination of the reduced forms of **8a** and **8b**, a first-order kinetics with a preceding acid−base equilibrium can be described as:

$$\frac{\mathrm{d[tryptamine]}}{\mathrm{d}t} = k\frac{K_a}{K_a + [H^+]}[\mathrm{reactant}] \qquad (2)$$

wherein k is the rate constant for the unprotonated form and K_a describes the acid dissociation constant of the anilinium salt. With K_a(anilinium) = $10^{-4.6}$ the overall rate constant is 0.025k at pH 3 and 0.996k at pH 7, i.e., compared with our reaction conditions the single benzyl elimination step should proceed nearly 40 times faster at neutral pH. As the required regeneration step for the disassembly of **12** and **17** is an addition of water (or OH^-) to the iminoquinone methide, it should likewise be accelerated at higher pH. Thus, it can be expected that also the multiple release properties of compounds **12** and **17** would be significantly enhanced at pH 7. Unfortunately, all attempts to determine the release kinetics of our model compounds at neutral pH failed due to a spontaneous precipitation of the reduced (amino) forms in aqueous solution. However, Shabat et al. recently reported on a similar PABC-based triple release linker that liberates three molecules of tryptophane in consecutive 1,6- and 1,4-benzyl elimination steps.[12] At pH 7.4 complete release of the reporter molecule was observed within 40 min after enzymatic activation supporting our assumption that the relevant benzyl eliminations occur with considerably higher reaction rates when carried out at neutral pH.

In summary, we have developed a first LSE model system (**17**) that enables an effective release of reporter/effector molecules upon activation of a trigger unit. By analyzing the elimination kinetics of the employed double release linker (**1**), we found the 1,6-benzyl elimination to occur much more rapidly

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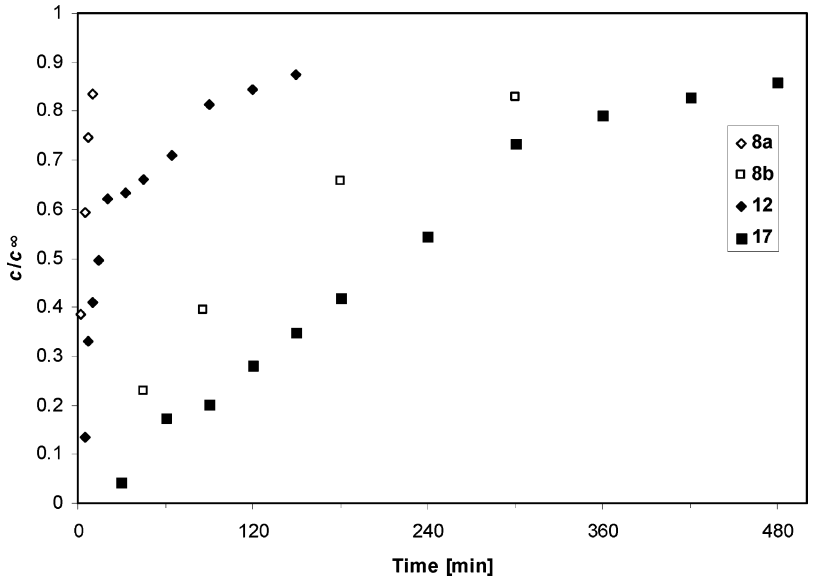

FIGURE 2. Release of tryptamine from **8a**, **8b**, **12**, and **17** after reduction with Zn/AcOH.

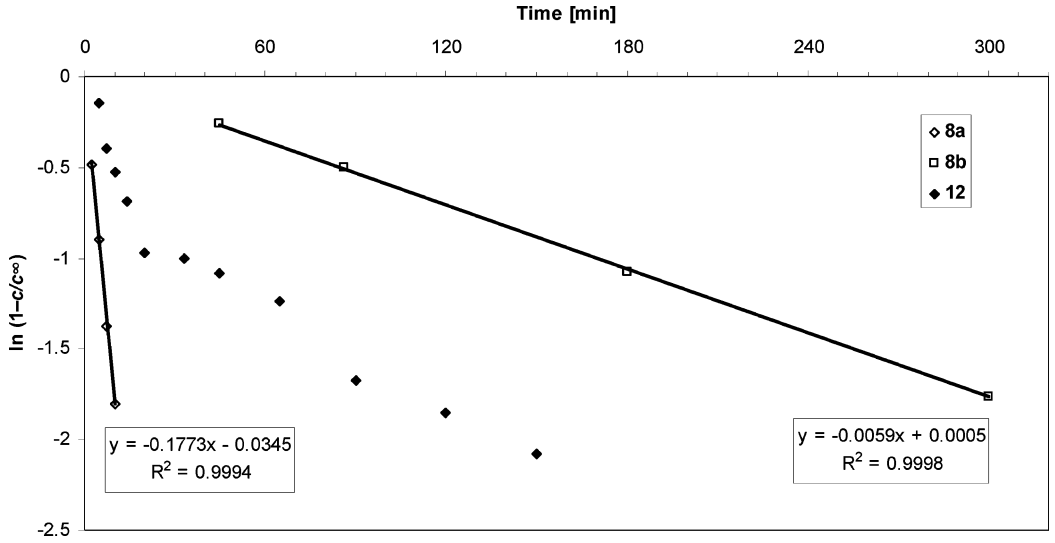

FIGURE 3. Release of tryptamine from **8a**, **8b**, and **12** after reduction with Zn/AcOH (logarithmic plot).

than the respective 1,4-elimination. Since both reactions proceed sufficiently fast, **1** proved to be a suitable building block for the construction of LSE systems. The synthetic strategy used for the preparation of **17**, however, is not feasible for the construction of longer and more complex LSE systems, especially, when aiming at combining different effector molecules or weakly reactive effectors such as aromatic amines or alcohols. A novel synthetic approach employing polymerizable linker-effector building blocks that circumvent the risk of any premature decomposition is currently under investigation.

Experimental Section

Ethyl 3-(Bromomethyl)-4-nitrobenzoate (2). A suspension of ethyl 3-methyl-4-nitrobenzoate (50.0 g, 239 mmol) and NBS (49.3 g, 277 mmol) in CCl_4 (600 mL) was heated for 30 min in a three-necked flask equipped with a Dean−Stark apparatus to remove any traces of water. After cooling and removal of the Dean−Stark apparatus, a 20-mg portion of $(BzO)_2$ was added and the reaction mixture was heated under reflux for 24 h. Addition of $(BzO)_2$ followed by 24 h of heating was repeated until TLC (1:4 EtOAc−hexane) indicated nearly complete conversion of the starting material. The solids were removed by filtration over celite then washed with DCM, and the filtrate was concentrated in vacuo. The dark brown residue was crystallized from EtOAc/hexane affording 44.29 g (64%) of pure **2** as a pale yellow solid: mp 105−106 °C (lit.[13] compound was obtained as an oil); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (t, J = 7.2 Hz, 3H), 4.43 (q, J = 7.1 Hz, 2H), 4.83 (s, 2H), 8.05 (d, J = 8.6 Hz, 1H), 8.12 (dd, J_1 = 1.8 Hz, J_2 = 8.4 Hz, 1H), 8.22 (d, J = 1.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 27.9, 62.1, 125.5, 130.6, 133.0, 133.6, 134.9, 150.4, 164.1; MS (CI^+ (NH_3)) m/z 208.1 ([M − Br]$^+$, 100), 305.1 (M + NH_4^+, 24).

Ethyl 3-(Hydroxymethyl)-4-nitrobenzoate (3). A suspension of **2** (10.0 g, 34.7 mmol) and Ag_2SO_4 (10.8 g, 34.7 mmol) in dioxane/water (1:1, 300 mL) was heated under reflux in the dark for 3 h. After cooling to room temperature, brine (150 mL) was added and stirring was continued for 10 min. The mixture was

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extracted with EtOAc (5 × 150 mL) and the combined organic layers were dried over $MgSO_4$. After removing the volatiles in vacuo, the residue was purified by flash chromatography (1:4 EtOAc−hexane) to yield 6.26 g (80%) of **3** as a colorless solid: mp 62−63 °C (lit.[13] compound was obtained as an oil); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (t, J = 7.1 Hz, 3H), 2.78 (br s, 1H), 4.42 (q, J = 7.0 Hz, 2H), 5.01 (s, 2H), 8.08 (s, 2H), 8.41 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 61.94, 61.96, 124.9, 129.4, 130.8, 135.1, 137.1, 149.8, 164.7; MS (CI^+ (NH_3)) m/z 243.2 (M + NH_4^+, 100).

Ethyl 3-((*tert*-Butyldimethylsilyloxy)methyl)-4-nitrobenzoate (4). A solution of **3** (5.65 g, 25.1 mmol) in anhydrous DMF (10 mL) was added dropwise within 30 min to a stirred solution of *tert*-butyldimethylchlorosilane (4.16 g, 27.6 mmol) and imidazole (2.56 g, 37.7 mmol) in anhydrous DMF (40 mL). After stirring at room temperature for 16 h, the reaction mixture was poured into water (400 mL) and extracted with DCM (3 × 100 mL). The combined organic layers were washed with water (2 × 100 mL) and dried over $MgSO_4$. After removing the volatiles in vacuo, the residue was recrystallized from methanol/water (6:1) affording 7.91 g (93%) of pure **4** as colorless needles: mp 77 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.15 (s, 6H), 0.99 (s, 9H), 1.42 (t, J = 7.2 Hz, 3H), 4.42 (q, J = 7.1 Hz, 2H), 5.10 (s, 2H), 8.05−8.08 (m, 1H), 8.12 (d, J = 8.3 Hz, 1H), 8.59−8.61 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.4, 14.2, 18.3, 25.8, 61.76, 61.81, 124.6, 128.6, 129.6, 134.9, 138.5, 148.8, 164.9; MS (CI^+ (NH_3)) m/z 326.2 (100), 340.2 (M + H^+, 49), 357.3 (M + NH_4^+, 23).

Ethyl 4-Amino-3-((*tert*-butyldimethylsilyloxy)methyl)benzoate (5). 4 (11.0 g, 32.4 mmol) and zinc powder (10 g) were suspended in DCM (500 mL) in a double-necked flask equipped with a condenser. To the stirred mixture was added acetic acid (18.4 mL, 0.32 mol) in DCM (100 mL) dropwise within 30 min and stirring was continued for 1 h. Excessive zinc powder was filtered off and the filtrate was washed with saturated $NaHCO_3$ solution (3 × 150 mL), dried over $MgSO_4$, and evaporated in vacuo to yield 10.0 g (100%) of **5** as a colorless solid: mp 47 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.07 (s, 6H), 0.89 (s, 9H), 1.36 (t, J = 7.2 Hz, 3H), 4.33 (q, J = 7.2 Hz, 2H), 4.67 (br s, 2 H), 4.71 (s, 2H), 6.62 (d, J = 8.3 Hz, 1H), 7.72 (d, J = 1.9 Hz, 1H), 7.80 (dd, J_1 = 2.0 Hz, J_2 = 8.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3, 14.4, 18.2, 25.8, 60.3, 64.8, 114.6, 119.2, 123.9, 130.3, 130.8, 150.7, 166.8; MS (CI^+ (NH_3)) m/z 178.1 ([M − OTBS]$^+$, 100), 195.1 ([M − OTBS + NH_3]$^+$, 33), 310.3 (M + H^+, 17).

2-((*tert*-Butyldimethylsilyloxy)methyl)-4-(hydroxymethyl)-aniline (6). A solution of **5** (6.90 g, 22.3 mmol) in anhydrous THF (250 mL) was cooled to −78 °C and a 1 M solution of DIBALH in DCM (66.9 mL) was added dropwise within 75 min. The reaction mixture was stirred for 24 h at −78 °C and then poured into 500 mL of a saturated solution of Rochelle salt. Subsequently, the mixture was extracted with diethyl ether (3 × 150 mL), the organic layers were dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by flash chromatography (1:2 EtOAc−hexane) to yield 4.63 g (75%) of **6** as a colorless solid: mp 49−50 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.08 (s, 6H), 0.90 (s, 9H), 3.25 (br s, 3 H), 4.54 (s, 2H), 4.68 (s, 2H), 6.65 (d, J = 8.0 Hz, 1H), 7.04 (d, J = 2.0 Hz, 1H), 7.09 (dd, J_1 = 2.0 Hz, J_2 = 8.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3, 18.2, 25.8, 64.8, 65.3, 115.7, 125.2, 127.9, 128.0, 130.3, 145.8; MS (ESI^+) m/z 267.5 (M^+, 100), 136.0 ([M − OTBS]$^+$, 73).

General Procedure for the Reaction of 4-Nitrophenoxycarbonyl-Activated Carbonates with Amines. To a mixture of HOBt (1.5 mmol, 230 mg), finely ground molecular sieve 4 Å (250 mg), and the amine (1.5 equiv per activated carbonate group) in 5 mL of anhydrous DMF was added the activated alcohol (1.5 mmol). After stirring for 16 h at room temperature, the molecular sieve was filtered off and the solvent was removed in vacuo. Further workup was performed as described for the individual compounds.

4-Nitrobenzyl 2-(1*H*-Indol-3-yl)ethylcarbamate (1,6-Elimination Model Compound 8a). 8a was obtained from **7a** and tryptamine following the general procedure. Flash chromatography of the crude product (1:2 EtOAc−hexane) afforded **8a** (98%) as a light yellow solid (purity (HPLC) 98%): mp 120 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.85 (t, J = 7.5 Hz, 2H), 3.26−3.34 (m, 2H), 5.17 (s, 2H), 6.97 (t, J = 7.8 Hz, 1H), 7.06 (t, J = 8.1 Hz, 1H), 7.15 (d, J = 2.3 Hz, 1H), 7.34 (d, J = 8.1 Hz, 1H), 7.50−7.55 (m, 2H), 7.57 (d, J = 8.5 Hz, 2H), 8.23 (d, J = 8.9 Hz, 2H), 10.82 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.3, 41.2, 63.8, 111.3, 111.4, 118.10, 118.12, 120.8, 122.6, 123.4, 127.1, 127.9, 136.1, 145.3, 146.8, 155.7; MS (ESI^+) m/z 362.1 (M + Na^+, 100), 340.1 (M + H^+, 49).

2-Nitrobenzyl 2-(1*H*-Indol-3-yl)ethylcarbamate (1,4-Elimination Model Compound 8b). 8b was obtained from **7b** and tryptamine following Procedure B. Flash chromatography of the crude product (1:2 EtOAc−hexane) afforded **8b** (86%) as a light yellow solid (purity (HPLC) 97%): mp 100−101 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.85 (t, J = 7.5 Hz, 2H), 3.25−3.32 (m, 2H), 5.37 (s, 2H), 6.97 (t, J = 7.6 Hz, 1H), 7.06 (t, J = 8.0 Hz, 1H), 7.15 (d, J = 2.1 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.50−7.63 (m, 4H), 7.78 (t, J = 7.6 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 10.82 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.3, 41.2, 61.8, 111.3, 111.4, 118.10, 118.13, 120.8, 122.6, 124.7, 127.1, 128.6, 128.9, 133.0, 134.0, 136.1, 147.0, 155.6; MS (ESI^+) m/z 362.1 (M + Na^+, 100), 340.1 (M + H^+, 30).

Double Release Model Compound 12. 12 was obtained from **11** and tryptamine following the general procedure. Flash chromatography of the crude product (1:1 EtOAc−hexane) afforded **12** (96%) as a yellow film (purity (HPLC) 97%,; purity (NMR) 93% (contains approximately 7% ethyl acetate as the major contamination)): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.78−2.87 (m, 4H), 3.22−3.32 (m, 4H), 4.99 (s, 2H), 5.07 (s, 2H), 5.31 (s, 2H), 6.91−6.99 (m, 2H), 7.01−7.08 (m, 2H), 7.10−7.15 (m, 2H), 7.28−7.39 (m, 5H), 7.50−7.55 (m, 2H), 7.44−7.54 (m, 4H), 7.65−7.71 (m, 2H), 8.22−8.28 (m, 2H), 9.38 (s, 1H), 10.79 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.35, 25.39, 41.14, 41.19, 61.6, 64.6, 64.7, 111.3, 111.44, 111.50, 118.06, 118.10, 118.11, 120.8, 122.5, 123.5, 127.09, 127.11, 127.9, 128.2, 128.5, 135.1, 136.1, 144.6, 146.9, 153.8, 155.9, 156.2; MS (ESI^+) m/z 705.2 (M + H^+, 100), 727.2 (M + Na^+, 48).

LSE System Model Compound 17. 17 was obtained from **16** and tryptamine following the general procedure. Flash chromatography of the crude product (3:2 EtOAc−hexane) afforded **17** (96%) as a pale yellow foam (purity (HPLC) 95%; purity (NMR) 93% (contains approximately 5% of ethyl acetate, 2% of DMF, and traces of *n*-hexane)): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.78−2.86 (m, 6H), 3.22−3.32 (m, 6H), 4.98 (s, 2H), 5.05 (s, 2H), 5.09 (s, 2H), 5.12 (s, 2H), 5.30 (s, 2H), 6.91−6.99 (m, 3H), 7.02−7.08 (m, 3H), 7.10−7.14 (m, 3H), 7.26−7.55 (m, 15H), 7.66−7.70 (m, 2H), 8.22−8.27 (m, 2H), 9.23 (s, 1H), 9.40 (s, 1H), 10.79 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.44, 25.49, 41.3, 59.7, 61.68, 61.73, 64.7, 64.9, 111.3, 111.55, 111.60, 118.17, 118.21, 120.9, 122.6, 123.6, 127.2, 128.0, 128.3, 128.5, 129.0, 133.1, 133.5, 135.3, 135.5, 136.2, 144.6, 147.0, 153.9, 154.2, 156.0, 156.23, 156.27; MS (ESI^+) m/z 1070.3 (M + H^+, 100), 1092.2 (M + Na^+, 40), 1108.2 (M + K^+, 11).

HPLC. Chromatographic conditions: Nucleosil C8 column (100−5, 250 × 4 mm) from Macherey-Nagel; flow: 1.0 mL/min; mobile phase A: 30% MeCN, 70% H_2O, 0.1% TFA; mobile phase B: 80% MeCN, 20% H_2O, 0.1% TFA; gradient: 0−5 min mobile phase A isocrat., 5−15 min 0% to 100% mobile phase B, 15−20 min 100% mobile phase B isocrat., 20−25 min 0% to 100% mobile phase A, 25−40 min mobile phase A isocrat.; injection volume: 50 μL; detection at 280 nm.

Cleavage Studies. Reduction with Zn/AcOH was performed by adding approximately 10 mg of zinc powder and 25 μL of glacial acetic acid to 475 μL of a 1 mM stock solution (acetonitrile) of the respective model compound. The mixture was vortexed for 1

min, excessive zinc was immediately filtered off by using a 0.2 μm syringe filter, and the resulting clear solution was diluted with tempered (37 °C) water/acetonitrile 9:1 to a final concentration of 60 μM. The samples were stored in a heating block (37 °C) and analyzed with HPLC at appropriate intervals. The relative degree of conversion was calculated by dividing the time-dependent tryptamine concentration c_t (from the peak area at the respective time) by the tryptamine concentration c_∞ at nearly complete conversion (peak area determined after 1.5 h for **8a** or 24 h for **8b**, **12**, and **17**, respectively).

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Supporting Information Available: Additional synthetic procedures, ^{1}H and ^{13}C NMR spectra of all compounds, as well as chromatograms of the model compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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