

New Synthesis and Characterization of (+)-Lysergic Acid Diethylamide (LSD) Derivatives and the Development of a Microparticle-Based Immunoassay for the Detection of LSD and Its Metabolites

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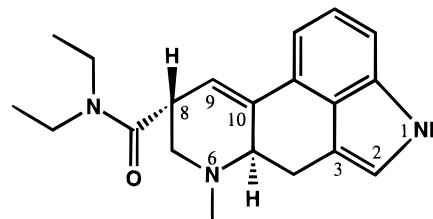
In this paper are reported the synthesis and characterization of three LSD derivatives. On the basis of several analytical characterization studies, the most stable derivative has been selected and a procedure to covalently link the derivative to polystyrene microparticles through a carrier protein has been developed. In addition, two new LSD immunogens have been synthesized and characterized, and from these immunogens antibodies that recognize not only LSD but also several major LSD metabolites have been generated. Using the selected derivative and antibody, a homogeneous microparticle-based immunoassay has been developed for the detection of LSD in human urine with the required sensitivity and specificity for an effective screening assay. The performance of this LSD OnLine assay has been evaluated using the criteria of precision, cross-reactivity, correlation to the Abuscreen LSD RIA and GC/MS/MS, assay specificity, and limit of detection.

INTRODUCTION

(+)-Lysergic acid diethylamide (LSD,¹ Scheme 1) is a highly potent drug that acts on the central nervous system and alters sensory perception, states of consciousness, and thought processes. By causing these altered states, the drug produces severe visual and auditory hallucinations. In addition to these physical effects, the use of LSD has been and continues to be a problem for drug and law enforcement agencies around the world (1–4). Making the problem even more complex is the fact that the detection of LSD in body fluids of users is difficult because the quantities typically ingested are very small (100–250 $\mu\text{g}/\text{dose}$) (4). To date, limited research has been conducted on the chemical properties of LSD. It is, however, known that LSD has an inherent fluorescence, which can be excited at 320 nm and emits at 445 nm. Additionally, under UV light irradiation, LSD can undergo catalytic hydration at the C-9,10 double-bond position. Once hydration occurs, the loss of fluorescence at 445 nm is observed. In alkaline solution, LSD undergoes an epimerization at the C-8 position, resulting in partial formation of iso-LSD. LSD is also unstable under prolonged heat exposure, but the mechanism of the thermal decomposition is not yet fully understood (5, 6).

Under physiological conditions, LSD is rapidly and extensively converted to several known and unknown

Scheme 1. Structure of LSD



metabolic products. At present, several metabolites of LSD in the human body, such as *N*-demethyl-LSD (nor-LSD), 2-oxo-3-hydroxy-LSD, 13-hydroxy-LSD, and 14-hydroxy-LSD, have been tentatively identified; yet only one metabolite (nor-LSD) and the parent compound, both excreted at 1% of the total dose, have been conclusively identified (6–10).

Currently, the measurements of LSD and its metabolites in biological fluids rely on radioimmunoassay methods or HPLC fluorescence methods and very specialized GC/MS/MS methods for confirmation (7–13). These methods produce undesirable radioactive waste or require extensive pretreatment of samples, very specialized equipment, and highly trained personnel. There has been much interest in recent years, because of the reported increased abuse, to develop nonisotopic, highly automated, homogeneous analytical methods to detect or screen for LSD abuse. Due to the lower sensitivities of nonisotopic immunoassays, the instability of LSD under both physiological and nonphysiological conditions, and the lack of information about the majority of the metabolites, the development of nonisotopic immunoassays to date has been a challenge. It is, therefore, extremely important to systematically synthesize LSD derivatives and study the stability of these derivatives for the development of a nonisotopic immunoassay. A conjugate procedure for making a LSD microparticle is also critical for obtaining a highly sensitive LSD assay. In addition, it is very important to generate antibodies that are capable of recognizing not only LSD but also its major metabolites. These antibodies must have low cross-

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¹ Abbreviations: BTG, bovine thyroglobulin; DMSO, dimethyl sulfoxide; DMF, *N,N*-dimethylformamide; CMC, *N*-cyclohexyl-*N*-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GC/MS/MS, gas chromatography/tandem mass spectrometry; KPi , potassium phosphate; $\text{NHB}\cdot\text{H}_2\text{O}$, *N*-hydroxybenzotriazole hydrate; NHS, *N*-hydroxysuccinimide; LSD, (+)-lysergic acid diethylamide; nor-LSD, *N*-demethyl-LSD; NMR, nuclear magnetic resonance; SAMHSA, substance abuse and mental health services administration; TEA, triethylamine; THF, tetrahydrofuran; TLC, thin-layer chromatography; TNBS, trinitrobenzenesulfonic acid.

reactivity to structurally related compounds that are not substances of abuse.

In this paper, we describe the synthesis and characterization of new LSD derivatives used in the development of a microparticle-based OnLine immunoassay. We also describe a new procedure to make a LSD microparticle. Like other OnLine immunoassays, this assay is based on the principle of the kinetic interaction of microparticles in solution (KIMS) (14), in which the drug content in the urine is directly proportional to the inhibition of the microparticle agglutination. Four LSD derivatives were synthesized, two of which (**3** and **7**, Scheme 3) were used to prepare immunogens (**4** and **8**, Scheme 3) for antibody production and three of which (**6**, **7**, and **10**, Schemes 2 and 3) were examined for best stability. From this work the most stable derivative was selected for preparing the conjugate. For the development of this microparticle-based assay, the selected LSD derivative was covalently coupled to a carrier protein, and this conjugate was then covalently linked to microparticles. These newly developed LSD microparticles, together with the properly selected antibodies, were then developed into a competitive displacement immunoassay for LSD with a detection limit of 0.2 ng/mL LSD.

MATERIALS AND METHODS

Reagents. All solvents were obtained from Fisher Scientific (Pittsburgh, PA) unless specified. All flash grade silica gel and silica gel preparative TLC plates were obtained from E. M. Science (Gibbstown, NJ). Protein concentrations were determined by using the Bradford protein assay reagents (15) purchased from Bio-Rad (Hercules, CA), and 2-oxo-3-hydroxy-LSD was purchased from Radian (Austin, TX). LSD, nor-LSD, 1-(3-aminobutyl)-*N,N*-diethyl-*D*-lysergamide, and 1-(3-aminopropyl)-*N,N*-diethyl-*D*-lysergamide (**1a** and **1b**, Scheme 2) (16) and Abuscreen RIA were prepared by Roche Diagnostic Systems (Somerville, NJ). LSD antibodies for the Abuscreen RIA were generated using a LSD analogue derivatized through the indole nitrogen and conjugated to BSA. Carboxylated polystyrene microparticles were obtained from Bangs Laboratories (Carmel, IN). CMC, NHB·H₂O, ovalbumin, and other reagents were obtained from Sigma (St. Louis, MO).

Instrumentation. Fluorescence measurements were carried out by using an LS-5B luminescence spectrometer (Perkin-Elmer, Norwalk, CT). The excitation wavelength was set at 320 nm, and the emission wavelength was measured at 445 nm. Light irradiation was performed using a 20 W desk-top fluorescent light. Proton nuclear magnetic resonance (¹H NMR) spectra were recorded at 400 MHz on an XL-400 NMR spectrometer (Varian, Palo Alto, CA); coupling constants are given in hertz (Hz), and CDCl₃ was used as the solvent. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; m, multiplet. The OnLine immunoassay was performed using an Olympus AU800 automated analyzer (Olympus, Lake Success, NY).

Synthesis of LSD Labels. *Synthesis of 1-(3-Aminobutyl)-*N,N*-diethyl-*D*-lysergamide and 1-(3-Aminopropyl)-*N,N*-diethyl-*D*-lysergamide (**2a** and **2b**, Scheme 2).* A solution of 900 mg (1.7 mmol) of **1a** in 25 mL of methanol was treated with 0.385 mL (12.3 mmol) of anhydrous hydrazine and stirred at room temperature overnight. The reaction mixture was concentrated at reduced pressure. The residue was treated with 25 mL of a mixture of 9:1 methylene chloride/methanol, and the insoluble solids were filtered off. The filtrate was chromatographed on 200 g of silica gel using 25% methanol

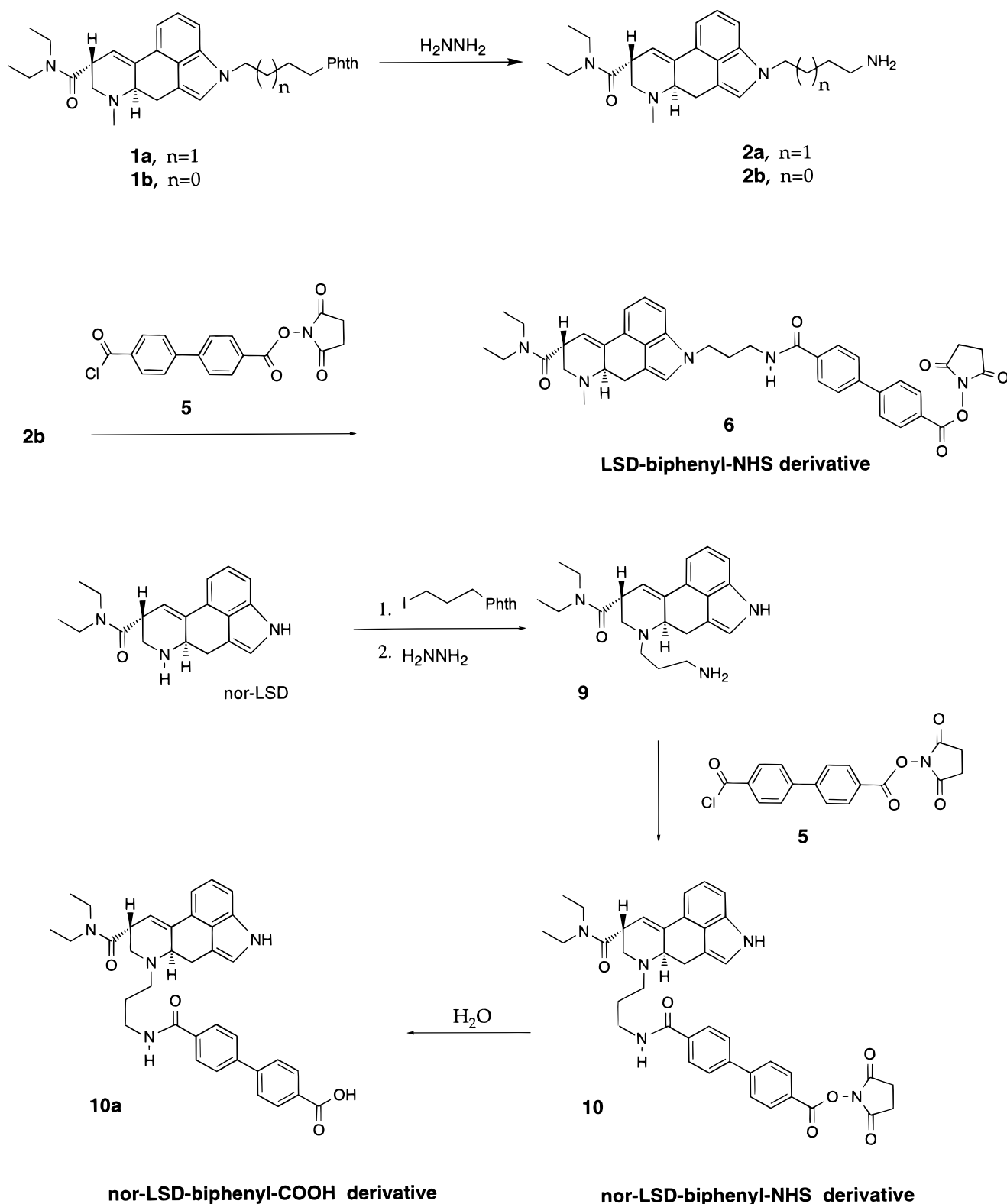
in methylene chloride as an eluent to elute front-running impurities, followed by 2% triethylamine/25% methanol in methylene chloride as an eluent to elute the product to yield 563 mg (83%) of **2a** as a yellow amorphous solid: ¹H NMR (400 MHz, CDCl₃) δ 1.17 (3H, t, *J* = 7.1), 1.24 (3H, t, *J* = 7.1), 1.50–1.60 (2H, m), 1.82–1.92 (2H, m), 2.59 (3H, s), 2.62–2.92 (6H, m), 3.02–3.10 (1H, m), 3.18–3.26 (1H, m), 3.37–3.57 (6H, m), 3.84–3.92 (1H, m), 4.08 (2H, t, *J* = 6.8), 6.33 (1H, s), 6.79 (1H, s), 7.11–7.20 (3H, m); MS, *m/e* 394 (M⁺); HR-EI MS calcd for M⁺ 394.2733, found 394.2731. Likewise, **2b** was obtained using an analogous procedure in 80% yield.

*Synthesis of 1-[[[4-Isothiocyanatophenyl]carbonyl]amino]butyl]-*N,N*-diethyl-*D*-lysergamide (**3**, Scheme 3).* A solution of 370 mg (0.94 mmol) of **2a** in 15 mL of anhydrous THF was cooled to 0 °C and treated with a solution of 190 mg (0.96 mmol) of 4-isothiocyanatobenzoyl chloride (17) in 5 mL of anhydrous THF and then stirred at 0 °C for 30 min and then at room temperature overnight; the reaction was driven to completion by adding 0.14 mL (1.0 mmol) of triethylamine and stirred at room temperature for 2 h. The reaction mixture was concentrated at reduced pressure. The residue was dissolved in methylene chloride, washed with H₂O, dried over anhydrous sodium sulfate, and concentrated at reduced pressure. The residue was chromatographed on 200 g of silica gel using 3% methanol in methylene chloride as an eluent to yield 325 mg (62%) of **3** as a tan amorphous solid: ¹H NMR (400 MHz, CDCl₃) δ 1.17 (3H, t, *J* = 7), 1.24 (3H, t, *J* = 7), 1.55–1.65 (2H, m), 1.80–2.05 (4H, m), 2.61 (3H, s), 2.63–2.75 (1H, m), 2.85–2.95 (1H, m), 3.04–3.12 (1H, m), 3.18–3.28 (1H, m), 3.35–3.58 (4H, m), 4.13 (2H, t, *J* = 6.4), 5.97–6.03 (1H, m), 6.35 (1H, s), 6.79 (1H, s), 7.10–7.20 (3H, m), 7.24 and 7.67 (4H, AA' BB'q, *J* = 8.4); MS, *m/e* 555 (M⁺); [α]_D = +47.5° (*c* 0.91%; CHCl₃).

*Synthesis of 4-[[[2,5-Dioxo-1-pyrrolidinyl]oxy]carbonyl]-[1,1'-biphenyl]-4-carbonyl Chloride (**5**, Scheme 2).* A mixture of 2.0 g (8.2 mmol) of 4,4'-biphenyldicarboxylic acid in 40 mL of anhydrous THF was treated with 5.0 mL (55.0 mmol) of oxalyl chloride followed by 0.02 mL of anhydrous DMF. The reaction was stirred at room temperature for 10 min and then heated to reflux for 90 min. The reaction was then concentrated at reduced pressure to a yellow oil. This was recrystallized from a mixture of THF and ether to yield 1.67 g (73%) of the diacid chloride as yellow needles: ¹H NMR (200 MHz, CDCl₃) δ 7.75 and 8.22 (8H, AA' BB'q, *J* = 8); MS, *m/e* 278 (M⁺).

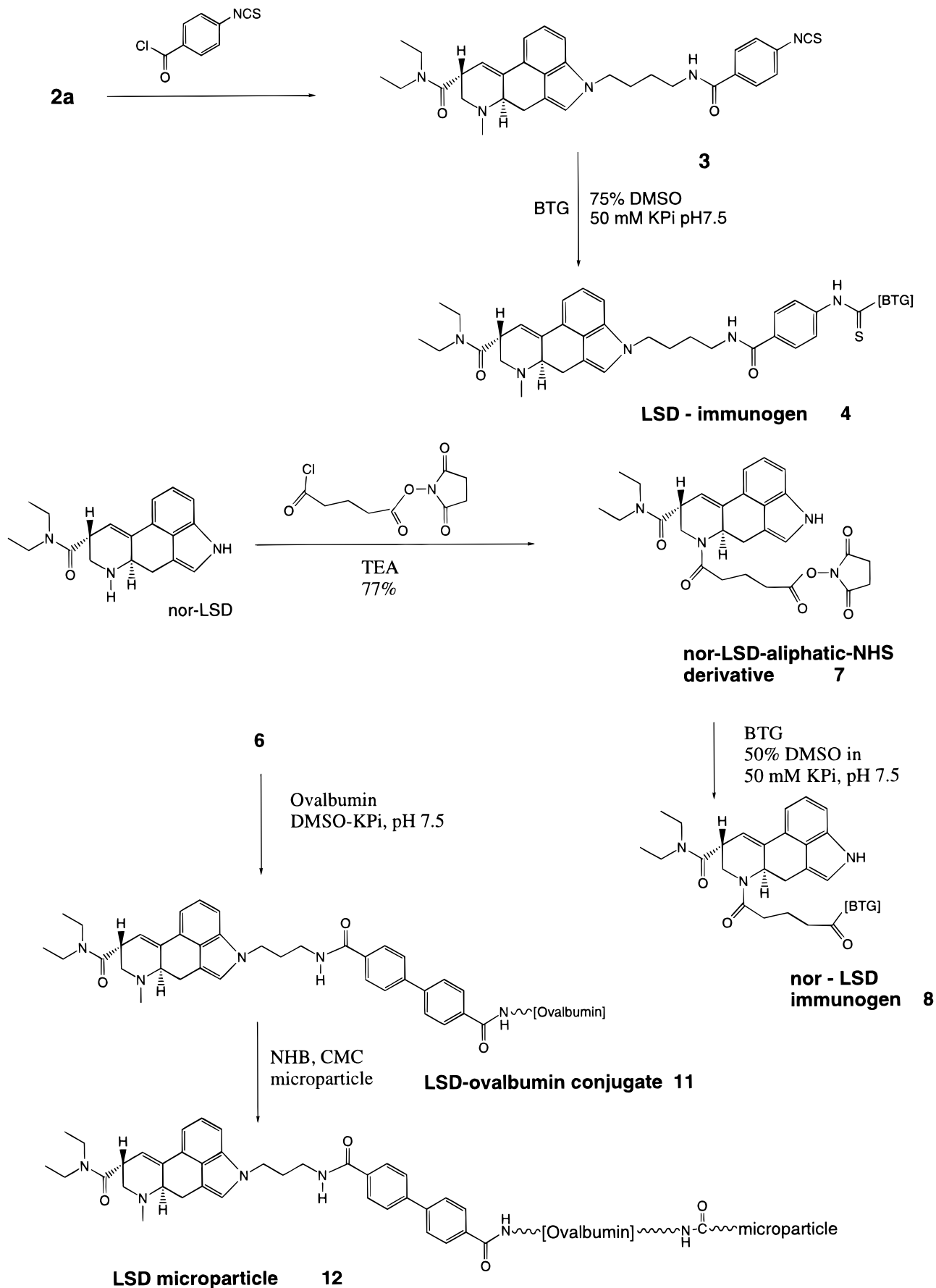
A solution of 1.67 g (6.0 mmol) of 1,1'-biphenyl-4,4'-dicarbonyl chloride in 65 mL of anhydrous THF was treated with 710 mg (6.17 mmol) of *N*-hydroxysuccinimide, followed by 0.835 mL (6.0 mmol) of triethylamine. The reaction was stirred at room temperature for 2 h, after which time it was filtered to remove triethylamine HCl. The filtrate was concentrated at reduced pressure to yield 2.0 g (93%) of **5** as a pale yellow solid: IR (CHCl₃) 1775, 1742 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 2.94 (4H, br s), 7.77 (4H, d, *J* = 8.5), 8.23–8.27 (4H, m); MS, *m/e* 357 (M⁺).

*Synthesis of 1-[3-[[[4-[[[2,5-Dioxo-1-pyrrolidinyl]oxy]carbonyl][1,1'-biphenyl]-4-yl]carbonyl]amino]propyl]-*N,N*-diethyl-*D*-lysergamide (**6**, Scheme 2).* A solution of 850 mg (2.375 mmol) of **5** in 65 mL of anhydrous THF under argon was cooled to 0 °C in an ice bath and then treated with a solution of 900 mg (2.365 mmol) of **2b** in 50 mL of anhydrous THF and 0.6 mL (4.3 mmol) of triethylamine added dropwise over a 20 min period. The reaction mixture was stirred at 0 °C for 1 h and then warmed to room temperature, with stirring, for 1 h. The mixture

Scheme 2. Synthesis of LSD Derivatives

was concentrated at reduced pressure, and the residue was dissolved in 100 mL of methylene chloride. The solution was washed with 100 mL of H_2O , 100 mL of saturated sodium bicarbonate solution, and 100 mL of saturated brine solution, dried over anhydrous sodium sulfate, and concentrated at reduced pressure to a brown residue. This was chromatographed on a short column of 100 g of silica gel using first methylene chloride as an eluent, then 9:1 methylene chloride/ether as an eluent to elute front-running impurities, and then 14:1 methylene chloride/isopropyl alcohol as an eluent to elute the

product to yield 650 mg (39%) of **6** as a cream-colored solid: IR (CHCl_3) 1772, 1743 cm^{-1} ; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.12–1.22 (6H, m), 2.18–2.22 (2H, m), 2.52 (3H, s), 2.60–2.68 (1H, m), 2.72–2.82 (1H, m), 2.93 (4H, s), 3.00–3.10 (2H, m), 3.38–3.58 (7H, m), 3.85–3.92 (1H, m), 4.24–4.32 (2H, m), 5.63–5.71 (1H, m), 6.41 (1H, s), 6.86 (1H, s), 7.20–7.25 (3H, m), 7.36 and 7.57 (4H, AA' BB' q, $J = 8.8$), 7.75 and 8.21 (4H, AA' BB' q, $J = 8.8$); MS, m/e 702 (M^+). An almost equal amount of the dimer was also obtained as a yellow oil: IR (CHCl_3) 3445, 2780, 1653, 1627 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3) δ 1.16 (3H, t, $J = 7.2$),

Scheme 3. Preparation of LSD Protein Conjugates

1.21 (3H, t, $J = 7.1$), 2.15–2.23 (2H, m), 2.51 (3H, s), 2.56–2.64 (1H, m), 2.77 (1H, t, $J = 11$), 2.96–3.03 (1H, m), 3.03–3.09 (1H, m), 3.35–3.47 (5H, m), 3.47–3.55 (2H,

m), 3.80–3.86 (1H, m), 4.20–4.30 (2H, m), 5.90–5.96 (1H, m), 6.85 (1H, s), 7.17–7.25 (3H, m), 7.45 and 7.54 (4H, AA' BB', $J = 8.1$); MS, m/e 967 (M^+ H).

Synthesis of 1-[[5-[8 β -9,10-Didehydro-8-[(diethylamino)carbonyl]ergolin-6-yl]-1,5-dioxopentyl]oxy]-2,5-pyrrolidinedione (7, Scheme 3). A solution of 200 mg (0.65 mmol) of nor-LSD in 10 mL of anhydrous THF, under argon, was treated with 161 mg (0.65 mmol) of 5-[[2,5-dioxo-1-pyrrolidinyl]oxy]-5-oxopentanoyl chloride (20, 21), followed by 0.2 mL (1.4 mmol) of anhydrous triethylamine. The reaction mixture was stirred at room temperature for 30 min and then concentrated at reduced pressure. The residue was dissolved in methylene chloride, washed with H₂O and saturated aqueous sodium bicarbonate solution, dried over anhydrous sodium sulfate, and concentrated at reduced pressure to yield 330 mg (98%) of **7** as a yellow amorphous solid: UV (CH₃-OH) λ_{\max} 308 ($\epsilon = 8980$); IR (KBr) 3396, 1814–1739, 1634, 1628 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.08–1.18 (3H, m), 1.25–1.38 (3H, m), 2.05–2.25 (2H, m), 2.43–3.25 (6H, m), 2.83 (4H, s), 3.28–3.60 (6H, m), [4.26 (d, $J = 13.6$) (major) and 5.02 (d, $J = 13.6$) (minor)] (1H, rotamers), [4.73–4.81 (minor) and 5.25–5.33 (major)] (1H, m, rotamers), 6.38 (1H, m), [6.90 (major) and 6.95 (minor)] (1H, s, rotamers), 7.08–7.30 (3H, m), 8.00 (1H, m); MS, m/e 521 (M⁺).

Synthesis of 8 β -6-(3-Aminopropyl)-9,10-didehydro-N,N-diethylergoline-8-carboxamide (9, Scheme 2). Alkylation of nor-LSD with iodopropylphthalimide was carried out according to the procedure of Marzoni and Garbret (16). The resulting phthalimide derivative (820 mg, 1.65 mmol) in 30 mL of methanol was treated with 0.4 mL (12.7 mmol) of anhydrous hydrazine and stirred at room temperature overnight. The reaction mixture was filtered and concentrated at reduced pressure. The residue was chromatographed on 100 g of silica gel using 2% triethylamine/15% methanol in methylene chloride as an eluent. Fractions containing product were combined and rechromatographed on 150 g of silica gel using 2% triethylamine/15% methanol in chloroform as an eluent to yield 560 mg (93%) of **9** as a yellow amorphous solid: IR (CHCl₃) 3479, 1663, 1624 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.17 (3H, t, $J = 7$), 1.25 (3H, t, $J = 7$), 1.70–1.80 (1H, m), 1.83–1.93 (1H, m), 1.90 (2H, s), 2.60–2.73 (2H, m), 2.79–2.87 (1H, m), 2.90–2.99 (2H, m), 2.99–3.09 (1H, m), 3.13–3.20 (1H, m), 3.25–3.33 (1H, m), 3.35–3.52 (6H, m), 6.28 (1H, s), 6.88 (1H, s), 7.10–7.17 (2H, m), 7.17–7.23 (1H, m), 8.20 (1H, br s); MS, m/e 366 (M⁺).

Synthesis of 1-[[[4'-[[[3-[8 β -9,10-Didehydro-8-[(diethylamino)carbonyl]ergolin-6-yl]propyl]amino]carbonyl]-1,1'-biphenyl]-4-yl]carbonyl]oxy]-2,5-pyrrolidinedione (10, Scheme 2). A solution of 1.32 g (3.7 mmol) of **5** in 50 mL of anhydrous methylene chloride under argon was cooled to 0 °C and treated with a solution of 535 mg (1.46 mmol) of **9** in 50 mL of anhydrous methylene chloride added dropwise over a 30 min period. After the addition was completed, the reaction mixture was washed with a saturated aqueous sodium bicarbonate solution, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was chromatographed on 100 g of silica gel using 5% isopropyl alcohol as an eluent. Fractions containing product were combined and concentrated at reduced pressure to a yellow solid. The solid was redissolved in ether and concentrated five times to remove residual isopropyl alcohol to yield 280 mg (28%) of **10** as a yellow solid: IR (CHCl₃) 3479, 1773, 1743, 1636 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.86 (3H, m), 1.11 (3H, t, $J = 6.9$), 1.82–1.92 (1H, m), 1.93–2.10 (1H, m), 2.70–2.88 (4H, m), 2.93 (4H, s) 3.08–3.20 (2H, m), 3.22–3.38 (4H, m), 3.40–3.60 (4H, m), 3.60–3.70 (1H, m), 3.98–4.08 (1H, m), 6.35 (1H, s), 6.93 (1H, s), 7.15–7.25 (3H, m), 7.61 and 7.95 (4H, AA' BB'q, $J = 8$), 7.67 and

8.19 (4H, AA' BB'q, $J = 8.3$), 8.00 (1H, s), 9.05 (1H, br s); MS, m/e 688 (M⁺H); [α]_D = +9° (c 0.355%, CHCl₃).

Preparations of Protein Conjugates. **Synthesis of 1-[[[4-Isothiocyantophenyl]carbonyl]amino]butyl]-N,N-diethyl-d-lysergamide-BTG (4, Scheme 3).** A solution of 698 mg of BTG in 20 mL of 50 mM KP_i, pH 7.5, was cooled to 0 °C and treated with 58 mL of DMSO, added dropwise, very slowly over a 2 h period. The mixture was treated with a solution of 90 mg (0.16 mmol) of **3** in 2 mL of DMSO, added dropwise very slowly. The reaction mixture was stirred at room temperature for 18 h, poured into a dialysis bag of 50 kDa molecular weight cutoff, and dialyzed 10⁸-fold in 50 mM KP_i, pH 7.5. The resulting conjugate was filtered through a 0.22 μ m sterile filter to yield 116 mL of the LSD-BTG immunogen **4**. The protein concentration was determined to be 5.3 mg/mL. The degree of drug substitution on the BTG protein was determined by the ability of remaining uncoupled lysine residues to react with TNBS (18, 19). Unmodified BTG, at the same concentration as the conjugate, was treated in the same manner with TNBS to provide a control. This procedure produced a yellow complex with an absorbance maximum at 325 nm and was used to calculate the drug substitution expressed as percent modification. The assay showed a 63.8% modification of available lysines on BTG.

Synthesis of 5-[8 β -9,10-Didehydro-8-[(diethylamino)carbonyl]ergolin-6-yl]-1,5-dioxopentyl-BTG (8, Scheme 3). A solution of 700 mg of BTG in 13 mL of 50 mM KP_i, pH 7.5, was cooled to 0 °C and treated with 13 mL of DMSO, added dropwise, very slowly. After the addition was complete, a solution of 84 mg (0.16 mmol) of **7** in 1 mL of DMSO was added dropwise very slowly. The reaction mixture was stirred at room temperature for 18 h, poured into a dialysis bag of 50 kDa molecular weight cutoff, and dialyzed 10⁶-fold in 50 mM KP_i, pH 7.5. The resulting conjugate was filtered through a 0.22 μ m sterile filter to yield the LSD-BTG conjugate **8**. The protein concentration was determined to be 12.1 mg/mL. The TNBS assay showed a 45% modification of available lysines on BTG.

LSD Derivatives Exposed to Fluorescent Light, Oxygen, and Different Solution pH Values. All LSD derivatives (**6**, **7**, and **10**) were placed in quartz cuvettes, and the irradiation experiments were conducted under the following conditions: LSD derivatives were dissolved in DMSO at a concentration of 1 mg/mL and further diluted to 2.6 mM in 10 mM KP_i buffer, pH 7.5, containing 0.09% NaN₃, 5 mM EDTA, and 0.1% Tween 20. The solutions were kept at 2–8 °C in the dark for 3 days to ensure a complete hydrolysis of the NHS ester (e.g. **10a**, Scheme 2). This is indicated by a complete disappearance of the starting material by TLC examination (data not shown). For the stability to light study, samples were exposed to a 20 W desk fluorescent light at room temperature. The distance between the fluorescent light source and the experimental samples was 15 cm. The experimental samples were subjected to irradiation for various times, and then fluorescence from the samples was measured, with $\lambda_{\text{ex}} = 320$ nm and $\lambda_{\text{em}} = 445$ nm. Triplicate measurements were performed under each condition. Coefficients of variation were found to be $\leq 1.5\%$ for the analytical method. From these data we established that $>9\%$ loss in fluorescent intensity represented significant decomposition of the tested compounds. This change of 9% represents three standard deviations from the mean, which is a $>99\%$ confidence interval. Corresponding LSD derivatives kept in the dark, at room temperature, were used as controls. These experiments were designed to investigate the effect of indoor light on the stability of the C-9,10 double-bond

position of LSD derivatives and to rank the light stabilities of these derivatives.

For stability to oxygen and solution pH studies, samples were placed in amber glass bottles and saturated with either oxygen or argon. Subsequently, these experimental samples were incubated at 45 °C for 10 days. Samples kept at 2–8 °C in the dark with argon were classified as the control. These experiments were conducted to explore the effect of oxygen, pH, and heat on the stability of the C-9,10 double-bond position of LSD derivatives and to rank the stability of these derivatives under these conditions.

Antibody Generation. Several goats were placed on a modified immunization program, as described by Vaitukaitis (22) using LSD immunogens (4 or 8). Briefly, immunogen 4 or 8 was mixed with Freund's adjuvant, and 1 mg of the immunogen containing complete Freund's was injected into multiple sites across the back of each goat. At week two, each goat continued to receive 1 mg of the immunogen containing incomplete Freund's. This injection was repeated twice at 1 week intervals, followed by a monthly injection of 0.5 mg of the immunogen mixed with incomplete Freund's adjuvant for a period of 6 months.

The individual animals were monitored for antibody titer and for cross-reactivity with LSD, nor-LSD, and 2-oxo-3-hydroxy-LSD by an ELISA method. Specifically, a selected derivative was covalently coupled to a carrier protein (ovalbumin). Polystyrene 96 well microtiter plates were coated with 50 μ L of a 1.6 μ g/mL LSD–ovalbumin conjugate in PBS buffer (50 mM KP_i , pH 7.2, containing 150 mM NaCl) and allowed to incubate for 2 h at room temperature or overnight at 2–8 °C. The plates were washed with PBS buffer and blocked with 1% BSA in PBS buffer. Fifty microliters of LSD, nor-LSD, or 2-oxo-3-hydroxy-LSD diluted in 1% BSA/PBS buffer at various concentrations or 50 μ L of 1% BSA/PBS buffer without the drug as a control was added into each well. Fifty microliters of the appropriate antiserum in 1% BSA/PBS buffer was then added to each well. The plates were incubated for 1 h at 37 °C and then washed with PBS/Tween 20 buffer. Anti-goat–alkaline phosphatase conjugate and *p*-nitrophenyl phosphate were then used to generate a detection signal. Two criteria were used to select antibodies: (1) affinity of antibodies as estimated by IC_{50} ; (2) inhibition of solid-phase antibody binding by soluble LSD and its major metabolites, namely, nor-LSD and 2-oxo-3-hydroxy-LSD. Once several animals were selected from an immunogen, a pool of antiserum was made and used to develop the immunoassay.

Preparation of LSD–Ovalbumin Conjugate (11, Scheme 3). Seventeen and a half milliliters of ovalbumin solution at 25 mg/mL in 50 mM KP_i buffer, pH 7.5, was cooled in an ice bath, and to this was slowly added 10 mL of DMSO. Seven and a half milligrams of selected LSD derivative was then dissolved in 1.5 mL of anhydrous DMSO to make a 5 mg/mL solution. The LSD derivative solution was added dropwise into the ovalbumin solution with stirring, and stirring was continued for 18 h at room temperature. The resulting LSD–ovalbumin conjugate was dialyzed 10¹²-fold using 30 kDa molecular weight cutoff dialysis bags. The final total protein concentration of LSD–ovalbumin conjugate was determined according to the Bradford protein assay (15).

Characterization of LSD–Ovalbumin Conjugate. To determine the concentration of the noncovalently bound LSD in the LSD–ovalbumin conjugate, the following experiments were conducted: 1.5 mL of 12.5 mg/mL LSD–ovalbumin conjugate in KP_i buffer, pH 7.5, was heat stressed at 45 °C in a white nontransparent poly-

ethylene container (HDPE) for 24 h. It was then immediately mixed with 1.5 mL of 40% DMF in 10 mM KP_i , pH 7.5. The material was then left at room temperature for 2 h, placed in a ovalbumin precoated Centricon filter (Amicon, Beverly, MA) with a molecular weight cutoff of 30 kDa, and centrifuged at 600*g* for 2 h. The resulting filtrate was then analyzed by fluorescence spectroscopy. Completely hydrolyzed LSD–biphenyl-NHS (6) solutions with concentrations ranging from 200 to 3200 ng/mL were used as fluorescence standard, and a linear regression method was used to generate a calibration curve. The concentration of LSD in the filtrate was derived from the standard curve. Filtrate from ovalbumin treated according to the same method as the LSD–ovalbumin conjugate was used as the control for background measurements.

A method was also designed to measure the total number of LSD derivatives per ovalbumin in the LSD–ovalbumin conjugate. The LSD–ovalbumin conjugate was diluted to a concentration of 0.125 mg/mL of total protein, and the fluorescence at 445 nm from the diluted conjugate solution was measured. Ovalbumin treated according to the same method as the LSD–ovalbumin conjugate was used as the control for a blank measurement. Completely hydrolyzed LSD–biphenyl-NHS (6) solution concentrations ranging from 200 to 3200 ng/mL were again used as fluorescence standards to generate a calibration curve. Total concentration of LSD molecules per ovalbumin was estimated from the calibration curve.

Preparation of the LSD Microparticle (12, Scheme 3). Ten milliliters of carboxyl-modified microparticle (10% solids) was first washed by centrifugation at 10000*g* with 0.1% Tween 20 in H₂O. To each milliliter of particles was added 20 mL of 0.1% Tween 20 in water, the mixture was centrifuged and decanted, and the particles were subsequently resuspended. This process was repeated five times, and the microparticle concentration was then adjusted to 3% (w/v) with a 0.1% Tween 20 solution. One and two-tenths milliliters of NHB (25 mg/mL, 0.37 mmol), previously dissolved in DMSO, was then added slowly to the 30 mL of microparticle suspension, under rapid stirring conditions, and the suspension was stirred for 10 min at 25 °C. To this suspension was added 1.7 mL of a freshly prepared CMC solution (50 mg/mL, 0.34 mmol), and the mixture was stirred slowly for 3 h at 25 °C. The material was then washed according to the method of centrifugation described above. The washed, activated microparticles (45 mL at 2%) were immediately mixed with LSD–ovalbumin/ovalbumin mixture at different molar ratios (total protein concentration was fixed at 3.1 mg/mL) diluted in 50 mM sodium bicarbonate buffer, pH 8.6, and this mixture was allowed to stir for 15 h at 25 °C. The resulting LSD microparticles were then washed according to the method of centrifugation described above using a wash solution of 10 mM KP_i buffer, pH 7.5, containing 0.09% NaN_3 and 0.1% Tween 20. The washed microparticle was then resuspended in this buffer at 1.0% solids (w/v).

Development of the LSD Immunoassay. The LSD immunoassay contains three reagents: (1) the antibody reagent, which was made by placing the titered antibody in a solution of 50 mM HEPES, pH 6.5, containing 0.1% BSA protein, 0.5% NaCl, and 0.09% NaN_3 ; (2) a reaction buffer containing 50 mM PIPES, pH 7.0, with 2–3% PEG, 2% NaCl, and 0.09% NaN_3 ; and (3) a LSD microparticle reagent, diluted from 1% stock solution to 0.2% solids in a buffer containing 10 mM KP_i , pH 7.5, 0.09% NaN_3 , and 0.1% Tween 20. In addition, LSD calibrators at concentrations between 0 and 1 ng/mL in normal urine containing 0.09% NaN_3 were used. The concentration of

these LSD standards were verified by GC/MS/MS method. The antibody concentration was adjusted so that the agglutination of the LSD microparticles was inhibited proportionally to the LSD concentration in the calibrators. The light scattering difference between different calibrators was also maximized in the calibration range (0–1 ng/mL) to obtain maximum sensitivity.

Cross-reactivity to structurally related compounds was conducted as follows: Normal human urine samples were spiked with the structurally related compound of LSD at various concentrations and tested as unknowns in the OnLine assay. The percent cross-reactivity of a structurally related compound was determined using the concentration of the compound that provided displacement equivalent to 0.5 ng/mL (1.5 nM) LSD.

One thousand presumed negative urine specimens were obtained from a large drug abuse screening laboratory. These samples had been previously screened and found to be negative for the SAMHSA five panel (cannabinoids, opiates, cocaine metabolite, amphetamine, and phencyclidine). At the time of analysis, these samples were simultaneously screened for LSD with the Abuscreen RIA and OnLine assay. The Abuscreen RIA has a >99.5% accuracy rate and was used as a reference method in addition to GC/MS/MS method. In addition, LSD positive samples were supplied by Dr. R. Foltz and Dr. D. Kuntz (Northwest Toxicology, Salt Lake City, UT). These samples had been previously screened positive by the Abuscreen RIA and were subsequently confirmed by GC/MS/MS. These samples were received frozen and were stored at -20°C until the day of analysis. For analysis, a qualitative screening assay was performed as follows: a single point calibration standard was used, and its absorbance value was assigned as the cutoff value. A positive result was reported if the sample absorbance value was greater than or equal to the absorbance of the cutoff calibrator.

RESULTS AND DISCUSSION

Synthesis and Stability Studies of the LSD Derivatives. A detailed analytical study of the synthesized LSD derivatives was necessary to determine which derivatives should be used to develop the LSD OnLine assay. The effect of exposure to fluorescent light on the LSD derivatives is shown in Figure 1. The C-9,10 double bond of LSD as previously reported can undergo photocatalytic hydration (5). A potential structure change or instability at this position was indicated by a change in fluorescence intensity when compared to a control. It was demonstrated that the fluorescence intensity of each compound decreased as the exposure time to fluorescent light increased. These results suggested that the order of stability of the LSD derivatives to photon-catalyzed hydration is LSD-biphenyl-COOH (hydrolyzed from **6**) > nor-LSD-biphenyl-COOH (hydrolyzed from **10**) > nor-LSD-aliphatic-COOH (hydrolyzed from **7**). It is hypothesized that the improved stability of **6** may come from the presence of biphenyl group at the N-1 position of the LSD molecule, where the biphenyl moiety can effectively stack on the LSD and exclude water molecules from interaction, thus stabilizing the photolabile C-9,10 center. Such hydrophobic stacking interaction has been documented (23, 24). When the biphenyl modification was at position 6 of the LSD molecule, the biphenyl linker could not effectively stack on the LSD and the protection efficiency was reduced. The aliphatic moiety at position 6 would be predicted to offer little or no protection effect to the LSD molecule, and indeed, this molecule had the least stability under these conditions. The LSD-biphenyl compound (**6**) was chosen for the construction of the

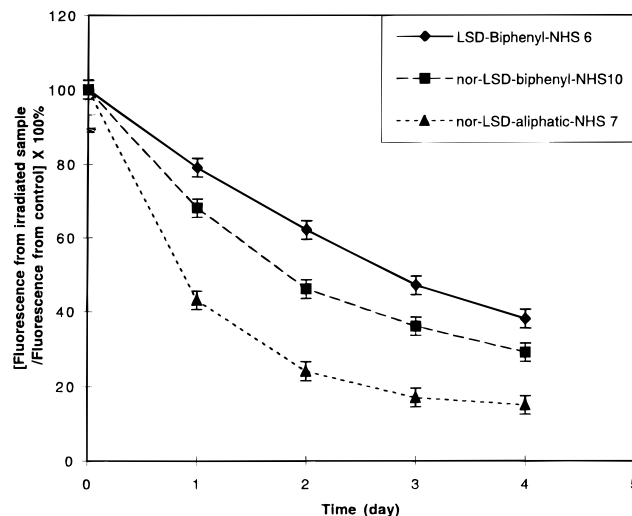


Figure 1. Effect of fluorescent light irradiation on the stability of LSD derivatives. Structural changes of LSD derivatives at the C-9,10 position were indicated by changes in fluorescence intensity. Condition: 2.6 mM LSD derivatives in 10 mM KPi, pH 7.5, buffer containing 5 mM EDTA, 0.09% NaN_3 , and 0.1% Tween 20. All experiments were conducted at 25°C . Corresponding LSD derivatives kept in the dark at 25°C were used as controls. The Y axis represents the fluorescence intensity of irradiated LSD derivatives when compared to their controls. The X axis is exposure time to fluorescent light. Error bar represents ± 2 SD.

conjugate used in the nonisotopic immunoassay. This compound displayed a 50–60% decomposition after having been exposed to the described conditions for 4 days. To ensure good assay stability, this issue was addressed by placing the microparticle reagent containing this derivative in a nontransparent polyethylene container. An accelerated stability study indicated that this container protects the reagent from light to ensure <10% loss at normal room light conditions for 1 year. Further data to emphasize the need not to have decomposition are reflected in cross-reactivity studies of antibodies generated from LSD immunogen (**4**) to completely photodecomposed LSD-biphenyl-COOH (**6**). It was found that the cross-reactivity of this decomposed material was <20% (data not shown), which would cause serious loss in assay sensitivity if the derivative was allowed to decompose.

Under heating conditions, oxygen may have an effect on the C-9,10 double bond (25). However, it appears from our experiments that oxygen did not affect the stability of the C-9,10 double bond for the LSD derivatives. Since protons can catalyze the hydration of the C-9,10 double bond (5, 25), we explored the effect of pH on the hydration of different LSD derivatives at high temperatures. Solution pH was chosen to be 6.0 or 7.5, because this is the most acceptable pH range for immunochemical reactions. At pH 6.0, in the presence or absence of oxygen, after 10 days at 45°C , compound **10** lost ~20% of its fluorescence intensity, while fluorescence intensity changes for compounds **6** and **7** were negligible. No significant changes in fluorescence intensity were observed for all three compounds when they were kept at pH 7.5 for 10 days at 45°C . The results suggested that the LSD derivatives were more stable in pH 7.5 buffer than in pH 6.0 buffer.

Synthesis of LSD Immunogen and the Generation of LSD Antibodies. Since only 1% of ingested LSD is excreted in urine and the typical ingested dose is very low, it is prudent to generate LSD antibodies that can recognize not only LSD but also LSD metabolites, yet avoid other undesirable ergot alkaloids compounds.

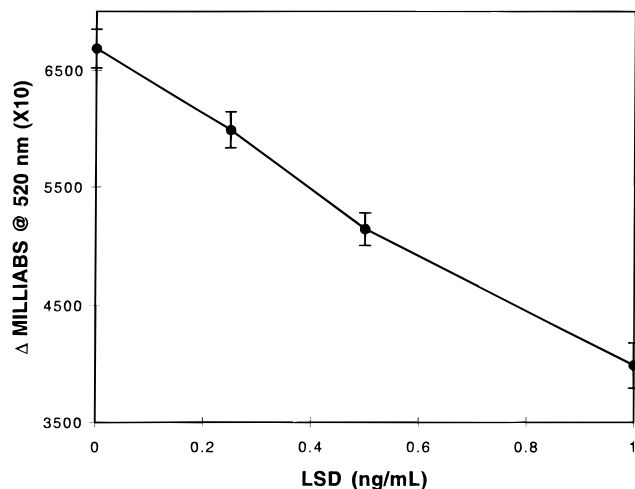


Figure 2. Representative concentration response curve on the Olympus AU800 analyzer of the Abuscreen LSD OnLine assay. Absorbance (ABS) is expressed as the milliabsorbance multiplied by a factor of 10. Error bar represents ± 1 SD.

Table 1. Qualitative Precision of Abuscreen LSD OnLine Assay^a

Within-run	Level 1	Level 2	Level 3	Level 4
LSD (ng/mL)	0.25	0.50	0.75	1.00
Mean ABS* (mA X 10)	6686	5989	5141	3985
SD (mA X 10)	165	154	140	193
CV %	2.5	2.6	2.7	4.9
Day-to-day	Level 1	Level 2	Level 3	Level 4
LSD (ng/mL)	0.25	0.50	0.75	1.00
Mean ABS* (mA X 10)	6587	5860	5156	3977
SD (mA X 10)	198	230	188	194
CV %	3.0	3.9	3.7	4.9

^a The Olympus AU 800 analyzer multiplies the milliabsorbance units by a factor of 10 to report results. Precision was determined to be $<3.0\%$ at 0 ng/mL (blank) urine.

Selectivity of an antibody to a hapten can be directed such that the antibody preferentially recognizes the part of the molecule that is farthest away from the attachment of the hapten to a carrier protein (26). It was assumed that this will allow the antibody to tolerate changes to the hapten near its point of attachment to the carrier protein. Under this hypothesis, we designed two immunogens (4 and 8). These immunogens directed antibodies to be less specific regarding changes near the indole ring of LSD (27) or at position 6 of LSD; therefore, cross-reactivity toward 2-oxo-3-hydroxy-LSD, 13-hydroxy-LSD, 14-hydroxy-LSD, and nor-LSD would be predicted to be higher for antibodies generated with immunogen 4. Antibodies generated from immunogen 8 would be expected to have high cross-reactivity toward 2-oxo-3-hydroxy-LSD and nor-LSD.

LSD-biphenyl-NHS (6) was used to construct the LSD-ovalbumin conjugate label for the ELISA method. The binding between the LSD-ovalbumin conjugate label and

Table 2. Cross-Reactivities of Abuscreen OnLine LSD Assay

Compound	Percent Cross-Reactivity	
	by weight concentration	by molar concentration
d-LSD	100	100
2-Bromo- α -ergocryptine	< 0.003	< 0.006
l-LSD (iso-LSD)	2.4	2.4
Lysergic acid N-(methylpropyl) amide	14	14
2-oxo-3-hydroxy-LSD	40	45
Lysergic acid N-(hydroxyethyl) amide	0.03	0.03
d-Lysergic acid	< 0.003	< 0.002
N-desmethyl-LSD (nor-LSD)	36	33
Methylsergide maleate	0.009	0.01
α -Ergocryptine	< 0.003	< 0.004
Ergotamine Tartrate	0.004	0.02
Ergonovine Maleate	0.004	0.005
Serotonin (5-hydroxy-tryptamine)	< 0.003	< 0.001
Tryptophan	< 0.003	< 0.002

the antibodies generated from immunogen 4 can be displaced with LSD (100%), nor-LSD (20%), and 2-oxo-3-hydroxy-LSD (50%). When antibodies generated from immunogen 8 were used, the binding between the LSD-ovalbumin conjugate label and antibodies could be displaced by LSD and had high cross-reactivity to nor-LSD (40%) but had $<20\%$ cross-reactivity to 2-oxo-3-hydroxy-LSD. From the cross-reactivity studies, antibodies raised against immunogen 4 were selected, and, from the derivative stability studies, derivative 6 was chosen for development of a LSD immunoassay.

Preparation and Characterization of LSD-Ovalbumin Conjugate. For a LSD assay, a 1:1 molar ratio between LSD-biphenyl-NHS (6) and ovalbumin was used to synthesize the LSD-ovalbumin conjugate stock solution. Since the LSD derivative is a highly hydrophobic compound, it can be trapped in ovalbumin or absorbed on the ovalbumin surface noncovalently. This unbound LSD derivative could be gradually released from the conjugate and could cause poor stability. The unbound LSD derivative could react with titrated antibody and reduce the sensitivity of the LSD assay. Therefore, it was important to develop a reproducible dialysis procedure to remove the unbound LSD from the LSD-ovalbumin conjugate. It was also important to establish a method to measure the noncovalently bound LSD derivatives on the conjugate so that methods could be selected that prevent this from occurring. To accomplish this, the conjugate was denatured at 45 °C and then in 20% DMF solvent to release any noncovalently bound LSD derivative. Under these mild denaturing conditions, no protein precipitation was observed. Our results indicated that free LSD derivative accounted for $<0.3\%$ of total LSD derivative loaded on ovalbumin when an extensive dialysis (10¹²-fold) procedure was used. This procedure was necessary to obtain a stable OnLine LSD assay reagent.

Methods were also developed to quantify the number of the LSD molecules per ovalbumin molecule in the

Table 3.

TOTAL SAMPLE CORRELATION		CHARACTERIZATION OF THE GC/MS/MS VALUES FOR THE LSD POSITIVE CLINICAL SAMPLES																	
<table border="1" style="margin: auto;"> <tr> <td colspan="2"></td> <td colspan="2" style="text-align: center;">ABUSCREEN RIA</td> </tr> <tr> <td colspan="2"></td> <td style="text-align: center;">+</td> <td style="text-align: center;">-</td> </tr> <tr> <td rowspan="2" style="text-align: center;">ABUSCREEN ONLINE</td> <td style="text-align: center;">+</td> <td style="text-align: center;">81</td> <td style="text-align: center;">7</td> </tr> <tr> <td style="text-align: center;">-</td> <td style="text-align: center;">0</td> <td style="text-align: center;">993</td> </tr> </table>				ABUSCREEN RIA				+	-	ABUSCREEN ONLINE	+	81	7	-	0	993	Mean Value for LSD: 0.8 ng/mL ISO-LSD: 1.2 ng/mL		
		ABUSCREEN RIA																	
		+	-																
ABUSCREEN ONLINE	+	81	7																
	-	0	993																
<table border="1" style="margin: auto;"> <tr> <td colspan="2"></td> <td colspan="2" style="text-align: center;">GC/MS/MS</td> </tr> <tr> <td colspan="2"></td> <td style="text-align: center;">+</td> <td style="text-align: center;">-</td> </tr> <tr> <td rowspan="2" style="text-align: center;">ABUSCREEN ONLINE</td> <td style="text-align: center;">+</td> <td style="text-align: center;">81</td> <td style="text-align: center;">0</td> </tr> <tr> <td style="text-align: center;">-</td> <td style="text-align: center;">0</td> <td style="text-align: center;">0</td> </tr> </table>				GC/MS/MS				+	-	ABUSCREEN ONLINE	+	81	0	-	0	0	Range LSD: 0.2-17.5 ng/mL ISO-LSD: 0.0-5.4 ng/mL		
		GC/MS/MS																	
		+	-																
ABUSCREEN ONLINE	+	81	0																
	-	0	0																
		Percentage of Samples in the Following Range:																	
		Range (ng/mL)	Sample Number	Percent															
		0.00-0.25	6	7.4															
		0.26-0.50	14	17.3															
		0.51-0.75	20	24.7															
		0.76-1.00	14	17.3															
		>1.00	27	33.3															

LSD-ovalbumin conjugate stock solution. Since amino groups at the protein surface are used in the coupling, normally, the degree of drug substitution can be determined by the ability of remaining uncoupled amine residues that react with TNBS. However, since the LSD substitution ratio was low (mean < one LSD per ovalbumin), poor results were obtained using the TNBS method. It was found that the number of LSD molecules in the conjugate could be estimated directly using the LSD fluorescence intensity. The fluorescence from ovalbumin at the same concentration as the conjugate was insignificantly small compared to the total intensity. Using completely hydrolyzed LSD-biphenyl-NHS (**6**) as a standard, the conjugates have been shown to contain 0.6–0.8 LSD molecules per ovalbumin molecule. Because coupling of LSD to ovalbumin would change the fluorescence quantum yield from LSD, this method only provided an estimate of the loading of LSD molecules per ovalbumin and was useful for quality control purposes (data not shown).

Synthesis and Characterization of LSD-Microparticle Conjugate. Several different molar ratios of the drug protein conjugate to microparticle were evaluated to determine the optimal ratio that produced the best dose response curve. In the development of a microparticle-based immunoassay, it is important that proper agglutination occurs in the absence of free antigen. To accomplish this, proper amounts of drug protein conjugate must be coupled to each microparticle such that an equivalence point can be reached, allowing the cross-linking of microparticles by antibody. Excess antigen or excess antibody in the system will prevent the formation of the large aggregates produced by cross-linking.

To establish the proper substitution of drug onto microparticle, LSD was first covalently coupled to the ovalbumin protein (stock conjugate) followed by mixing of the LSD-ovalbumin conjugate with ovalbumin at

various molar ratios and coupling the LSD-ovalbumin/ovalbumin mixture to the microparticle. Each coupled microparticle was then titrated against the antibody to determine the performance of each LSD-ovalbumin/ovalbumin molar ratio. The ratio that gave the greatest dose response curve and the lowest nonspecific binding (agglutination rate in the absence of antibody) was selected. This was determined to be a molar ratio of 1:8 (LSD-ovalbumin/ovalbumin).

Development of LSD Assay. Using an endpoint analysis reading at 520 nm, a dose response curve was generated with various concentrations of LSD as shown in Figure 2. The light scattering difference measured by light transmission from 0 ng/mL to the cutoff concentration of LSD (0.5 ng/mL) was > 130 milliabsorbances (mA); the overall difference from 0 to 1.0 ng/mL was > 240 mA. Table 1 shows that the qualitative intra-assay ($n = 20$) and interassay ($n = 100$) precision had CVs of < 5%.

Table 2 illustrates the cross-reactivity of the LSD OnLine assay to structurally related compounds of LSD. As expected, this assay had low cross-reactivity to iso-LSD (2.4%, molar concentration); the cross-reactivity to nor-LSD was 25% and to 2-oxo-3-hydroxy-LSD was 32%. The cross-reactivities of other structurally related compounds that are undesirable to detect, such as serotonin, tryptophan, ergotamine, egonovine, and others, were < 0.002%. Finally, the limit of detection (LOD) of the assay was determined by performing 20 replicate assays on the 0 ng/mL calibrator. Two standard deviations below the mean yields a LOD of < 0.2 ng/mL LSD.

Table 3 shows the correlation of the OnLine LSD screening assay with RIA and GC/MS/MS methods. GC/MS/MS confirmed LSD positive clinical samples were used to study patient correlation. Eighty-one positive samples were tested in the OnLine LSD assay. The distribution of LSD concentration in these samples is also shown in Table 3. Twenty percent of the samples

contained <0.5 ng/mL of LSD according to GC/MS/MS data. Due to the high cross-reactivity to major LSD metabolites, all of these samples were positive by OnLine LSD assay. One thousand presumptive negative samples were also tested; 993 were negative and 7 were positive. All of the presumptive negative samples were found to be negative by RIA. These seven samples that were OnLine positive and RIA negative were found to be GC/MS/MS negative.

CONCLUSION

A homogeneous microparticle-based immunoassay has been developed for the detection of LSD in human urine with the required sensitivity and specificity. Three major issues were considered when this assay was developed: (1) the stability of the LSD derivatives; (2) the stability of LSD microparticles; (3) the cross-reactivity of antibodies. Light, temperature, and solution pH can alter the structure of LSD at the C-9,10 double-bond position. Therefore, it is desirable to prepare and select a derivative that generates a stable LSD microparticle which is able to withstand long-term storage. On the basis of stability studies, we have selected LSD-biphenyl-NHS (**6**) as the best derivative for the development of the LSD OnLine assay. Besides a stable LSD derivative, LSD microparticles free of noncovalently bound LSD are necessary for the OnLine technology to obtain the required assay sensitivity and reagent stability. The conjugation and dialysis procedures reported here have allowed us to minimize unbound LSD in the LSD microparticle reagent and to achieve the targeted sensitivity and stability of the immunoassay. Due to the extent of LSD *in vivo* metabolism and low ingestion dosage, the concentration of parent compound (LSD) in urine is extremely low. To overcome this, we designed and selected an immunogen using a LSD analogue derivatized through the indole nitrogen and conjugated the derivative to BTG. The antibody generated by this immunogen has demonstrated broad reactivity toward LSD and several LSD metabolites. All of these factors were essential in the successful development of the LSD OnLine assay, which demonstrated excellent clinical sensitivity.

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