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SYNTHESIS OF PHOTODEPROTECTABLE SERINE DERIVATIVES. "CAGED SERINE"

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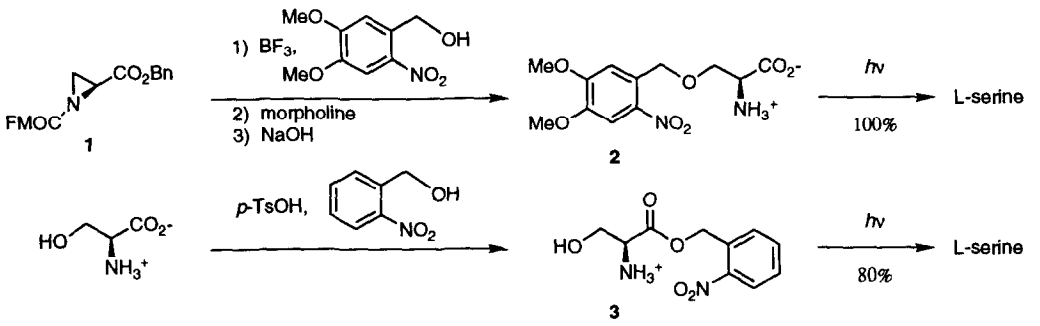
Abstract: The synthesis of O-(4,5-dimethoxy-2-nitrobenzyl)serine has been accomplished by treatment of an FMOC-aziridinecarboxylate with nitroveratryl alcohol followed by hydrolysis. Irradiation with a Pyrex-filtered 450W Hanovia lamp releases serine with a half-life of 4.4 min. *o*-Nitrobenzyl serinate is prepared by direct esterification. Vycor-filtered irradiation releases serine with a half-life of 6.9 min.

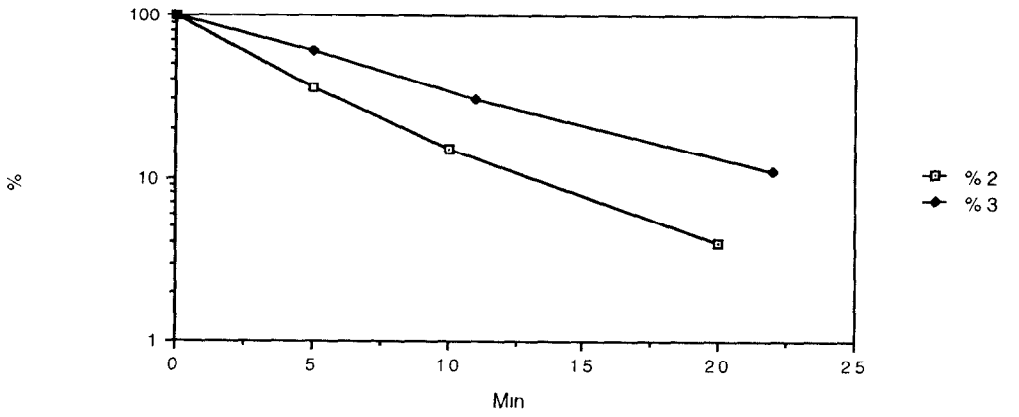
Recent advances in time-resolved techniques for studying biological processes[1] have been made possible by the availability of biomolecules bearing photochemically-removable protecting groups[2] Using these molecules, biochemical transformations including metal ion release and uptake can be triggered by light after an inactive effector has been placed in a favorable location.[3] The applications of this technology have even extended to reactions within enzyme crystals.[4] For the purpose of studying enzymes using serine as a substrate,[5] we require a photodeprotectable serine derivative. Because of the broad previous applications of nitrobenzyl groups in "caging" biomolecules, they were chosen for the protection of the serine hydroxyl and carboxyl. The dimethoxy analog was used for ether protection, while the parent system served for ester protection. Photodeprotectable serines with N-nitrobenzyl carbamate and O-nitroveratryl ester functionalities have recently been prepared for the study of amino acid receptors.[6]



Following several unsuccessful attempts to prepare an ether-caged serine using benzyl alkylating agents, the synthesis of (*S*)-O-(4,5-dimethoxy-2-nitrobenzyl)serine (**2**) was achieved by modification of the method of Okawa.[7] (*S*)-Serine is the starting material for the preparation of benzyl (*S*)-N-(fluorenylmethoxycarbonyl)aziridinecarboxylate (**1**) in a 6-step sequence (esterification, tritylation, sulfonation,

ring closure, detritylation, acylation). For closure of the aziridine ring, Okawa *et al.* report that either the mesylate or tosylate is suitable, but in our hands the latter is superior. Because of protecting group incompatibilities in subsequent steps, the group used by Okawa is substituted by an FMOC group in **1**. Alcoholysis of **1** occurs in 51% yield. Removal of the FMOC is accomplished with morpholine, and the benzyl ester is hydrolyzed in 76% yield overall. Experimental descriptions are provided in the Notes.[8]

Figure: Photodeprotection of Caged Serines



The deprotection of **2** was conducted at 3 mM concentration in degassed 28 mM $NaHSO_3$ buffer (pH 5.5) with a Pyrex-filtered 450 W Hanovia lamp ($\lambda > 300$ nm), following the reaction by NMR. Analysis of the rate of starting material loss under steady-state irradiation gives a half-life of 4.4 min (Figure). After 30 min of irradiation, serine is isolated by ion-exchange chromatography in quantitative yield. It was converted to its methyl ester ($SOCl_2$/MeOH) and derivatized with MTPA-Cl to establish its greater than 90% *ee* by NMR.[9] Several different buffer systems were examined for this deprotection, but only bisulfite provided a reaction clean enough to follow by NMR and to isolate the product. Presumably, bisulfite serves to inactivate the nitrosoaldehyde byproduct.

The preparation of the nitrobenzyl ester **3** was accomplished in a straightforward way in 62% yield using *p*-TsOH/CH_2Cl_2. The deprotection of this derivative (450 W Vycor-filtered Hanovia, $\lambda > 230$ nm) was conducted at 8 mM in 30 mM phosphate buffer (pH 7), following the reaction by NMR. Contrasted with the ether-caged serine, this reaction was straightforward to monitor and clean. As shown in the Figure, the half-life for deprotection of this ester is 6.9 min, significantly faster than the buffer-catalyzed background hydrolysis rate.

Further details of the kinetics of deprotection of these serine derivatives will be reported with the individual macromolecules under study. These results serve to emphasize that, despite the many studies that use nitrobenzyl photochemistry, the reactions are highly variable. Superior photoremovable groups are clearly needed.

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8. **Benzyl (*S*)-N-(9-fluorenylmethoxycarbonyl)aziridinecarboxylate (1).** A solution of 944 mg (5.3 mmol) of freshly prepared benzyl (*S*)-aziridinecarboxylate[6] in 30 mL benzene was added dropwise to a solution of 1.38 g (5.3 mmol) FMOC-Cl in 45 mL benzene cooled to 5 °C. The solution was stirred for 15 min, and then 615 μL (5.3 mmol) 2,6-lutidine was added. The solution was stirred for an additional 20 min and then partitioned between ether and water. The phases were separated, and the aqueous layer extracted twice more with ether. The combined ether layers were washed with brine and dried over Na_2SO_4. The solvent was removed by rotary evaporation. Flash chromatography on silica gel (hexane/EtOAc, 9/1) provided 1.84 g (87%) of a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (d, J=7.5 Hz, 2H), 7.57 (d, J=7.5 Hz, 2H), 7.41-7.22 (m, 9H), 5.14 (d, J=12.7 Hz, 1H), 5.14 (d, J=12.7 Hz, 1H), 4.41 (dd, J=7.3, 10.5 Hz, 1H), 4.28 (dd, J=7.1, 10.5 Hz, 1H), 4.17 (dd, J=7.1, 7.3 Hz, 1H), 3.11 (dd, J=3.1, 5.5 Hz, 1H), 2.62 (dd, J=1.0, 3.1 Hz, 1H), 2.43 (dd, J=1.0, 5.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.0, 160.7, 143.4, 141.20, 141.18, 134.8, 128.5, 128.4, 127.7, 127.0, 125.1, 119.9, 68.5, 67.6, 46.7, 34.9, 31.3; IR ($CDCl_3$): 3068, 2957, 1735 cm^{-1}; MS m/*e* calc'd for $C_{25}H_{21}NO_4$: 399.1470, found 399.1466; $[\alpha]_D^{20}$ -41.6° (c=0.54, CH_2Cl_2).

Benzyl (*S*)-N-(9-fluorenylmethoxycarbonyl)-O-(4,5-dimethoxy-2-nitrobenzyl)serinate. To a solution of 899 mg (2.25 mmol) **1** and 1.44 g (6.75 mmol) 4,5-dimethoxy-2-nitrobenzyl alcohol in 40 mL dry,

ethanol-free $CHCl_3$ at 5 °C was added a solution of 25 μL BF_3•etherate in 1 mL $CHCl_3$. The solution was stirred for 3 h and then quenched with aqueous $NaHCO_3$. The layers were separated, and the aqueous layer extracted twice with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. Silica gel (5 g) was added and the solvents were removed by rotary evaporation. The mixture was loaded directly onto the top of a silica gel column and eluted with hexane/EtOAc (8/2) to provide 703 mg (51%) of the triprotected serine as a pale yellow oil. Further elution allowed recovery of excess nitroveratryl alcohol, which could be recycled. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (d, J=7.5 Hz, 2H), 7.69 (s, 1H), 7.58 (d, J=5.8 Hz, 2H), 7.38 (t, J=7.5 Hz, 2H), 7.34-7.21 (m, 7H), 7.05 (s, 1H), 5.71 (d, J=8.5 Hz, 1H), 5.27 (d, J=12.3 Hz, 1H), 5.16 (d, J=12.3 Hz, 1H), 4.91 (d, J=15.4 Hz, 1H), 4.80 (d, J=15.4 Hz, 1H), 4.68-4.64 (m, 1H), 4.46 (dd, J=7.0, 10.5 Hz, 1H), 4.36 (dd, J=7.1, 10.5 Hz, 1H), 4.21 (dd, J=7.0, 7.1 Hz, 1H), 4.04 (dd, J=2.9, 9.3 Hz, 1H), 3.94 (s, 3H), 3.90 (dd, J=2.8, 9.3 Hz, 1H), 3.82 (s, 3H); IR ($CDCl_3$): 3434, 1729, 1513, 1273 cm^{-1}, MS m/*e* calc'd for $C_{34}H_{32}N_2O_9$: 612.2108, found 612.2095; $[\alpha]_D^{20}$ -8.2° (c=0.73, $CDCl_3$).

Benzyl (*S*)-O-(4,5-dimethoxy-2-nitrobenzyl)serinate. The triprotected serine (530 mg, 0.87 mmol) was stirred for 1 h at 0 °C with 40 mL morpholine. The solvent was then removed by rotary evaporation. Flash chromatography on silica, eluting with hexane/EtOAc (6:4) provided the dibenzofulvene/morpholine adduct. Further elution with MeOH/EtOAc (2:8) provided 266 mg (78%) of the free amine as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70 (s, 1H), 7.36-7.26 (m, 5H), 7.18 (s, 1H), 5.24 (d, J=12.3 Hz, 1H), 5.16 (d, J=12.3 Hz, 1H), 4.93 (d, J=15.7 Hz, 1H), 4.87 (d, J=15.7 Hz, 1H), 3.96 (dd, J=5, 9 Hz, 1H), 3.95 (s, 3H), 3.90 (s, 3H), 3.87 (dd, J=4, 9 Hz, 1H), 3.77 (t, J=3.9 Hz, 1H), 1.82 (br s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.6, 153.7, 147.3, 138.6, 135.4, 130.4, 128.4, 128.2, 127.8, 109.1, 107.6, 73.0, 69.8, 66.7, 56.25, 56.18, 54.9; IR (neat): 3387, 3322, 2933, 1737, 1514 cm^{-1}; MS m/*e* calc'd for $C_{19}H_{22}N_2O_7$: 390.1427, found 390.1416; $[\alpha]_D^{20}$ -24.8° (c=1.1, $CDCl_3$).

Sodium (*S*)-O-(4,5-dimethoxy-2-nitrobenzyl)serinate (2). To a solution of the ester (266 mg, 0.68 mmol) in 18 mL MeOH/H_2O (5/1) at 0 °C was added dropwise over 5 min 0.67 mL 1.0 N NaOH. The solution was stirred for 90 min at 0 °C, and then solvents were removed by rotary evaporation. The yellow solid was washed several times with ether to provide 213 mg (97%) of **2**. m.p. 149-151° (dec); R_F 0.53 (H_2O:EtOAc:HOAc:BuOH, 1:1:1:1); ^{1}H NMR (300 MHz, 100 mg/mL, D_2O): δ 7.25 (s, 1H), 6.92 (s, 1H), 4.63 (d, J=15.4 Hz, 1H), 4.59 (d, J=15.4 Hz, 1H), 3.83 (s, 3H), 3.73 (m, 2H), 3.69 (s, 3H), 3.47 (t, J=4.3 Hz, 1H); ^{13}C NMR (75 MHz, 100 mg/mL, D_2O): δ 179.9, 153.1, 146.5, 138.1, 130.4, 109.5, 107.4, 73.9, 69.6, 56.2, 55.9, 55.7; IR (KBr): 3376, 2942, 1585, 1522, 1275 cm^{-1}; UV-Vis (H_2O): λ_{max} 216 (4.02), 243 (3.94), 310 (3.65), 346 (3.75) nm; MS (FAB) m/*e* calc'd for $C_{12}H_{16}N_2O_7Na$ (MH+): 323.0855, found 323.0848; $[\alpha]_D^{20}$ 0 ± 1° (c=2.6, H_2O).

2-Nitrobenzyl serinate hydrotoluenesulfonate. A solution of L-serine (500 mg, 4.76 mmol), 2-nitrobenzyl alcohol (5.14 g, 23.8 mmol), and *p*-toluenesulfonic acid monohydrate (4.5 g, 23.6 mmol) in 60 mL chloroform was refluxed through a Soxhlet extractor containing molecular seives for 18h. The solvent was removed by rotary evaporation and the product precipitated with ether. Filtration and washing of the crystals with ether provided 1.21 g (62%) of the title compound. ^{1}H NMR (300 MHZ, D_2O): δ 8.16 (d, J=8.0 Hz, 1H), 7.78-7.57 (m, 5H), 7.32 (d, J=8.1 Hz, 2H), 5.64 (s, 2H), 4.37 (t, J=3.8 Hz, 1H), 4.12 (dd, J=4.2, 12.5 Hz, 1H), 4.03 (dd, J=3.4, 12.5 Hz, 1H), 2.36 (s, 3H); IR (KBr): 3390, 2913, 1752, 1517, 1198 cm^{-1}. UV-Vis (H_2O): λ_{max} 220 (4.19), 263 (3.77) nm, MS (FAB) m/*e* calc'd for $C_{10}H_{13}N_2O_5$: 241.0824, found 241.0830; $[\alpha]_D^{20}$ -7.3° (c=1.05, H_2O)

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