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Synthesis of N-Protected Serine Ethers as Precursors of New Photoactivatable Amino Acids Cleavable by Hydrogenolysis

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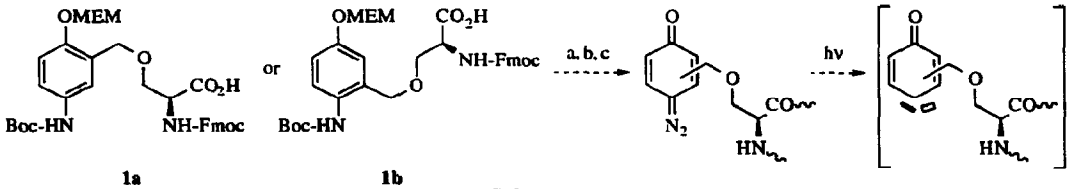
Abstract: Fmoc-(L)-serine ethers bearing either 2-(2'-methoxyethoxymethyl ether)-5-terbutyloxycarbonylaminobenzyl (ortho) or 5-(2'-methoxyethoxymethyl ether)-2-terbutyloxycarbonylaminobenzyl (meta) moieties were synthesized. Spectral characteristics of the corresponding photoactivatable 4-diazocyclohexa-2,5-dienones were determined.

Among the wide diversity of probes designed for studying proteins, photoactivatable peptides are useful tools for the structural exploration of receptors and enzymes.[1] Photoreactive hydrophobic amino acids such as parabenzoylphenylalanine[2] and paraazidophenylalanine[3] were extensively employed in photoaffinity labeling. However, until now, no hydrophilic amino acid has been used in photolabelling experiments. We designed a novel series of photoactivatable residues bearing a 4-diazocyclohexa-2,5-dienone (DCD)[4] moiety with moderate steric hindrance and mild hydrophilicity.[5] DCD strongly absorb at 350 nm ($\varepsilon > 25{,}000$ $Mol^{-1}.cm^{-1}$). Upon irradiation, they readily decompose without rearrangement into a highly reactive carbene which irreversibly inserts into neighbouring groups including C-H bonds.[4] Furthermore, DCD can be photolyzed by a tryptophan mediated energy transfer process which considerably improves the selectivity of labelling.[6] In this paper, we wish to report on the synthesis of serine ethers bearing a DCD moiety (DCD-Ser) as new photoactivatable amino acid residues (scheme 1).



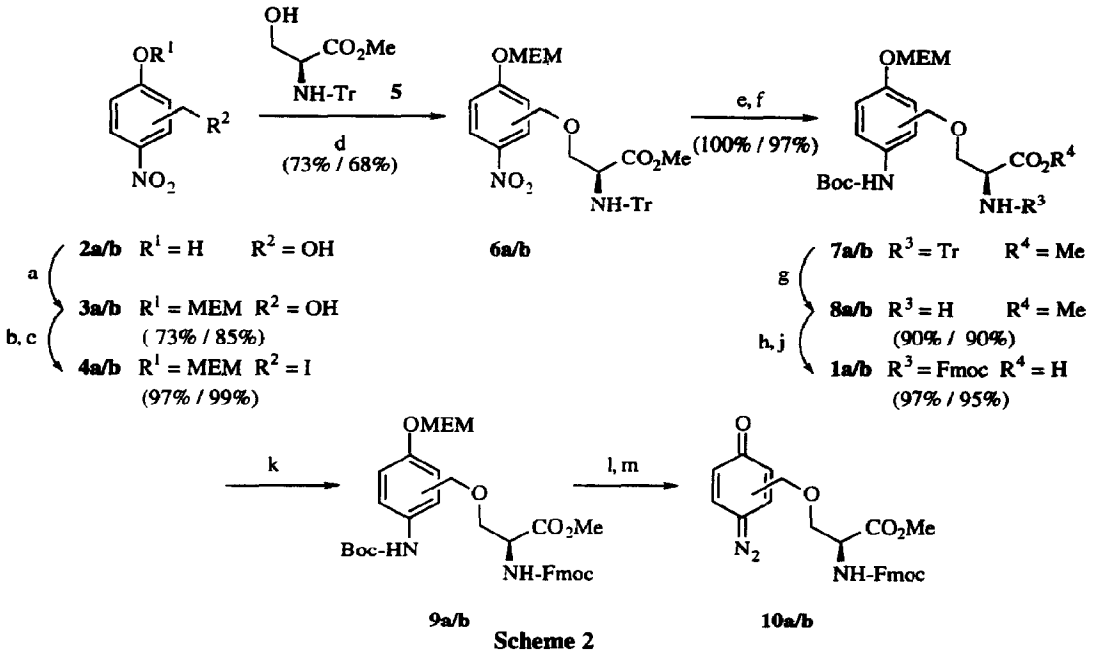
1a 1b

Scheme 1

a: peptide synthesis; b: deprotection: c: diazotization

Insertion of a photoactivatable group in a peptide via a benzyl ether bond is a valuable strategy for introducing tritium in the label : after irradiation, the protein-coupled benzyl moiety could be separated from the peptidic probe by tritiation after protein denaturation.[7] This should avoid an ambiguous sequence determination of the labelled area due to the peptidic nature of the probe. In addition, it should allow to bypass the synthesis of radioactive peptides.

Due to the sensitivity of DCD to light and strong nucleophiles, DCD-based amino acids are not consistent with standard solid phase peptide synthesis. For this purpose, acido-sensitive MEM and Boc derivatives of 2-hydroxy-5-aminobenzyl ether **1a** and 5-hydroxy-2-aminobenzyl ether **1b** are convenient precursors of DCD containing peptides. The synthesis of **1a/b** was achieved respectively in 48 and 50 % overall yields by the route depicted in scheme 2.

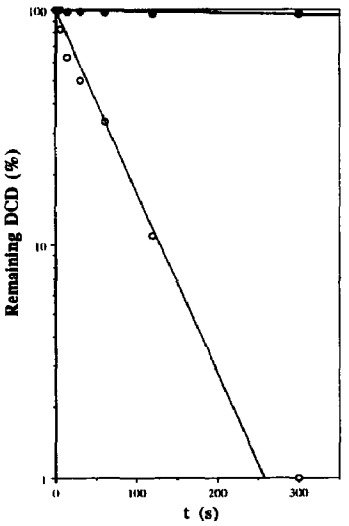


Scheme 2

a: NaH (1.05 equiv.) / DMF 50°C; MEMCl (1 equiv.) / THF. r.t.; b: CH_3SO_2Cl, NEt_3 / THF; c: NaI (4 equiv.) / acetone; d: 10% NaOH, Bu_4NHSO_4 (1.4 equiv.) / CH_2Cl_2; e: H_2 4.5 HPa, Lindlar / AcOEt-MeOH 1h rt; f: Boc_2O / MeOH 16 h; g: HCO_2H / $ClCH_2CH_2Cl$; h: LiOH / CH_3CN, H_2O; j: FmocNSu / THF; k: CH_2N_2; l: TFA- H_2O 9-1, 25°C 1 h; m: 0.1M $NaNO_2$ / TFA-H_2O 9-1, 4°C.

The starting material was 2-hydroxy-5-nitrobenzyl alcohol[8] **2a** and 5-hydroxy-2-nitrobenzyl alcohol **2b**. Protection of the phenols as methoxyethoxymethyl ethers[9] **3a/b** (NaH in DMF, 50°C, 30 mn then MEM Chloride in THF) and two step activation of the benzyl alcohols provided iodides **4a/b**.[10] Benzyl ethers **6a,/b** ($[\alpha]_D^{25}$ = +39° / $[\alpha]_D^{25}$ = +21°; C = 1, $CHCl_3$) were obtained by a phase transfer reaction using tetrabutylammonium hydrogen sulfate.[11] In contrast, O-alkylation of serine **5** in anhydrous conditions failed (LDA or NaH in THF or THF:HMPA 90:10) or proceeded very slowly (NaH in DMF, overnight; < 5%). The nitro group was selectively reduced by medium pressure hydrogenation with Lindlar catalyst. Protection of the highly oxydizable anilines was carried out in situ under hydrogen with diterbutyldicarbonate and afforded **7a/b** ($[\alpha]_D^{25}$ = +28° / $[\alpha]_D^{25}$ = +24°; C= 1, $CHCl_3$) in almost quantitative yields. Compounds **7a/b** were deprotected as follows: we ascertained that the bulky trityl group protected the methyl esters from the saponification.[12] As a consequence, saponification of the methyl ester necessitated first acidolysis of the triphenylmethyl moiety.[13] Treatment without purification of the amine by Fmoc-NSu gave the N-protected serines derivatives **1a** and **1b**.[14]

The spectral characteristics of the corresponding DCD were determined using methyl esters **10a/b** as models. These were prepared by quantitative esterification of **1a/b** followed by simultaneous deprotection of Boc and MEM groups in TFA; one pot diazotization (0.1 M $NaNO_2$, 4°C) and purification by HPLC afforded pure DCD **10a** and **10b**.[15] Their spectral characteristics were determined either in a Tris HCl buffer (0.1 M, pH 7.6) or in octanol as a mime of hydrophobic environment. Results summarized in table 1 are consistent with previous reports.[4,6] Compounds **10a/b** are stable several hours in the dark : less than 10% was degraded after 16 hours at 25°C in Tris buffer; satisfactory half-lifes were determined at 40°C. As a general rule, hydrophobic environment protects DCD against spontaneous degradation. Both compounds were rapidly photolysed when irradiated at 350 nm and no significant difference between ortho and meta isomer could be detected (figure 1).



Solvents	Tris HCl (0.1M, pH 7.6)		Octanol	
compounds	**10a**	**10b**	**10a**	**10b**
λ_{max} (nm)	351	352	355	356
ε ($Mol^{-1}.cm^{-1}$)	33,000	32,000	30,000	30,000
$t_{1/2}$ (dark, 25°C)	> 24 h	> 24 h	ND	ND
$t_{1/2}$ (dark, 40°C)	4.4 h	6 h	5.2 h	8.5 h
$t_{1/2}$ (350 nm)	29 s	37 s	40 s	40 s

table 1: Spectral characteristics of 10a/b and half-life times in the dark (T = 40°C) and when irradiated at 350 nm (C = 5.10^{-5} M, E = 10^{-2} $mW.cm^{-2}$, T = 10°C).

figure 1: Photolysis of **10b** in a Tris HCl buffer (0.1 M, pH 7.6) in the dark (T = 40°C), (o) and at 350 nm (T = 10°C), (o).

Diazotization of parahydroxyaniline was also carried out in the presence of an excess of lysine (8 equivalents relative to p-hydroxyaniline, C = 0.8 M): on the basis of spectral and HPLC data no nitrosation of lysine was observed when only 1.0 equivalent $NaNO_2$ was employed. This result suggests that DCD can be generated from the corresponding p-hydroxyaniline without affecting primary amines of the peptidic support.

Interesting properties of these compounds has prompted us to incorporate **1b** into a 29 amino acid peptidic membrane-spanning probe which study is in progress: compound **1b** has shown to be fully consistent with automatic Fmoc peptide synthesis.

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7. Model benzyl ether (Boc-Ser(-Bn)-OH) was quantitatively deprotected by hydrogenation (4.5 HPa, Pd-$CaCO_3$ / MeOH:H_2O, overnight) in presence of 0.1% SDS crude protein extracts.
8. **4a** was obtained quantitatively by treatment of the commercial 2-hydroxy-5-nitrobenzaldehyde by ethanolic sodium borohydride.
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1a. $[\alpha]_D^{25}$ = +6.1° (C = 1, CH_3COCH_3); ^1H NMR (C_3D_6O) δ (ppm) 8.23 (broad s, 1H), 7.84 (d, J = 7.5 Hz, 2H), 7.72 (d, J = 7.1 Hz, 2H), 7.39 (m, 7H), 7.08 (d, J = 8.7 Hz, 1H), 6.67 (broad d, J = 8.4 Hz, 1H), 5.22 (s, 2H), 4.58 (s, 2H), 4.52 (m, 1H), 4.35 (m 2H), 4.28 (m, 1H), 4.01 (dd, J = 4.6 Hz, J' = 9.7 Hz, 1H), 3.81 (m, 3H), 3.50 (m, 2H), 3.27 (s, 3H), 1.47 (s, 9H); MS (EI): m/z = 637. Anal. calcd: C, 64.15; H, 6.29; N, 4.40. Found: C, 64.52; H, 5.94; N, 4.11.
1b. $[\alpha]_D^{25}$ = +9.5° (C = 1, CH_3COCH_3); ^1H NMR (C_3D_6O) δ (ppm) 7.88 (d, J = 7.5 Hz, 2H), 7.76 (d, J = 7.2 Hz, 2H), 7.68 (broad, 1H), 7.62 (d, J = 8.8 Hz, 1H), 7.38 (m, 4H), 7.09 (d, J = 2.8 Hz, 1H), 7.00 (dd, J = 2.8 Hz, J' = 8.8 Hz, 1H), 6.85 (broad d, J = 8.3 Hz, 1H), 5.24 (s, 2H), 4.62 (s, 2H), 4.56 (m, 1H), 4.36 (m, 2H), 4.29 (m, 1H), 3.98 (dd, J = 4.7 Hz, J' = 9.7 Hz, 1H), 3.82 (m, 3H), 3.52 (m, 2H), 3.29 (s, 3H), 1.49 (s, 9H); MS (EI): m/z = 637. Anal. calcd: C, 64.15; H, 6.29; N, 4.40. Found: C, 64.54; H, 6.76; N, 4.50.
15. Corresponding p-hydroxydiazonium trifluoroacetate salts:
10a. λ_{max} = 316 nm, ε = 18,000 $Mol^{-1}.cm^{-1}$; ^1H NMR (C_3D_6O) δ (ppm) 8.62 (d, J = 9.6 Hz, 1H), other aromatic signals hidden by Fmoc.
10b. λ_{max} = 317 nm, ε = 18,000 $Mol^{-1}.cm^{-1}$; ^1H NMR (C_3D_6O) δ (ppm) 8.54 (d, J = 2.0 Hz, 1H), 8.33 (dd, J = 2.0 Hz, J' = 8.3 Hz, 1H), 8.11 (d, J = 8.3 Hz, 1H).

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