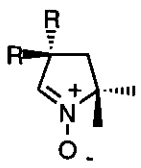
NEW DERIVATIVES OF PYRROLINE *N*-OXIDES AS SPIN TRAPS[1]

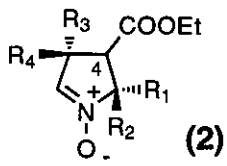
Prabhat Arya

Steacie Institute for Molecular Sciences, National Research Council Canada, Ottawa, Ontario, K1A 0R6, Canada

Abstract - Pyrroline-*N*-oxides containing maleimido (**4**), biotin (**5a**) and ether side chain (**6** and **7**) at the C-4 position have been synthesized starting from a common synthon (**8**).

Free radicals such as hydroxyl radicals and superoxide radical anions have been proposed as mediators in a variety of cellular responses, such as phagocytosis, ischemia/ reperfusion injury, aging and cancer.[2] Despite major efforts to study the role of these radicals in initiating tissue injury, the identification of these reactive species remains a major problem. Spin trapping technique combined with electron spin resonance (ESR) spectroscopy offers an opportunity to simultaneously measure and characterize these oxygen centered free radicals.[3] In this technique, transient free radicals are trapped by nitrone or nitroso compounds to give a persistent nitroxide spin trapped adduct that can be observed using a conventional ESR spectrometer. Among several spin traps, 5,5-dimethylpyrroline-*N*-oxide (M_2PO, **1a**), 3,3,5,5-tetramethylpyrroline-*N*-oxide (M_4PO, **1b**) are the commonly used spin traps.[4-6] A short and flexible synthetic strategy for obtaining modified M_4PO derivatives (**2** and **3**) was developed at the NRC.[7-9] This strategy not only allowed the introduction of different alkyl groups at C-3 and C-5 but also incorporated a carbethoxy group at the C-4 position. Introduction of the functional group at C-4 position is important because it can further be used for derivatization. Spin traps (**2** and **3**) were used as scavengers for many of the free radicals such as t-BuO$^\bullet$, $^\bullet CH_2OH$, $^\bullet$OH, Ph$^\bullet$ and the adducts resulting from the trapping reaction had half-lives of several hours.[7-9]





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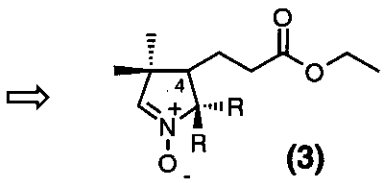


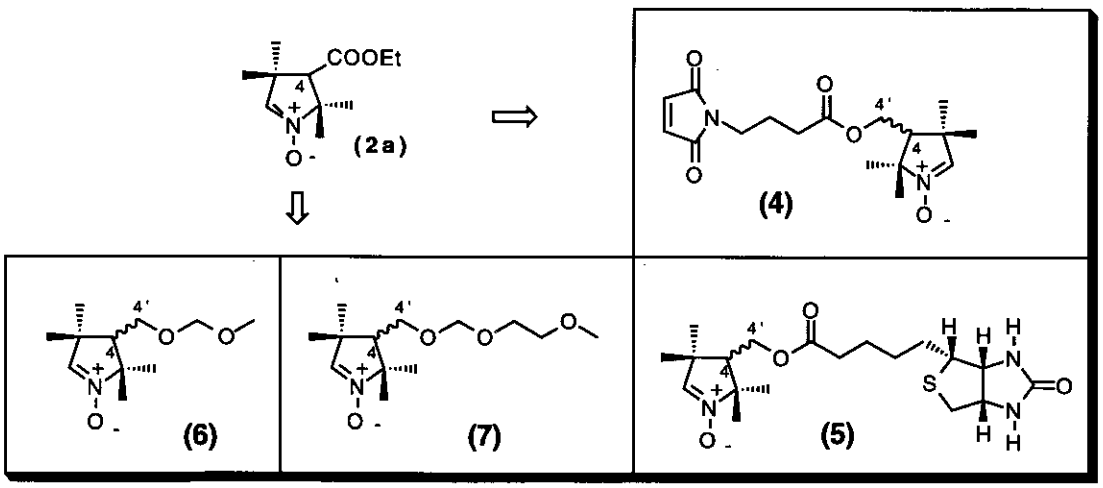
(3)

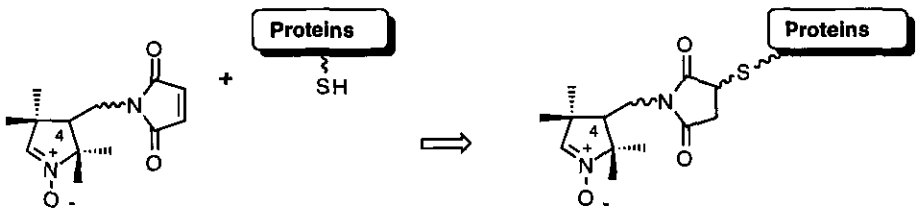
(1a) R = H, M_2PO

(1b) R = Me, M_4PO

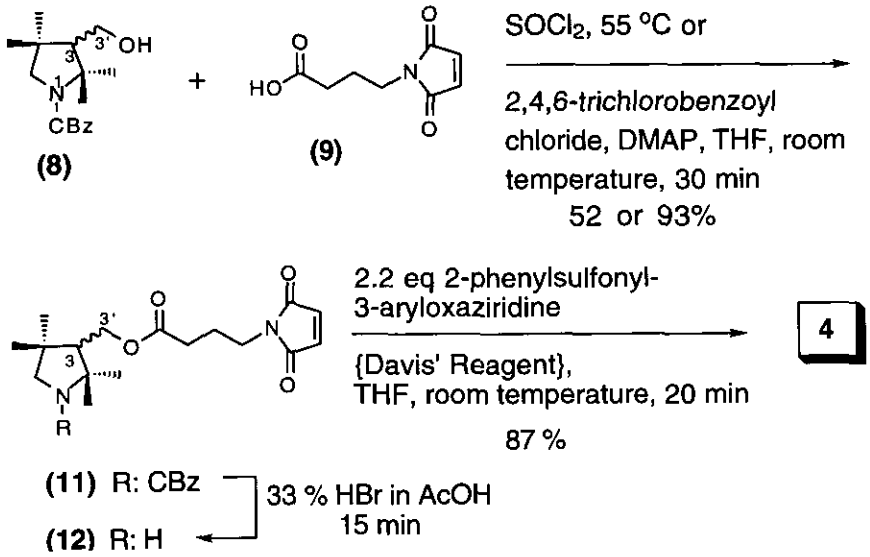
	R_1	R_2	R_3	R_4	R
(a)	CH_3	CH_3	CH_3	CH_3	CH_3
(b)	CD_3	CD_3	CH_3	CH_3	CD_3
(c)	CH_3	Ph	CH_3	CH_3	
(d)	CH_3	$(CH_2)_{11}CH_3$	CH_3	CH_3	
(e)	$-(CH_2)_5-$		$-(CH_2)_5-$		

In this paper, we are reporting our synthetic strategy in obtaining modified M_4PO derivatives having maleimido **(4)**, biotin **(5)** and the ether side chain **(6** and **7)** at the C-4 position. High affinity of the maleimido group towards nucleophilic attack can help the spin trap in cross linking the spin trap to a wide variety of proteins containing sulfhydryl groups as nucleophiles. Biotin binds strongly to proteins such as *avidin and streptavidin* and may be used as a marker on the spin trap. Addition of ether derivatives on the side chain of the spin trap may assist in enhancing the lipophilicity of the spin trap.

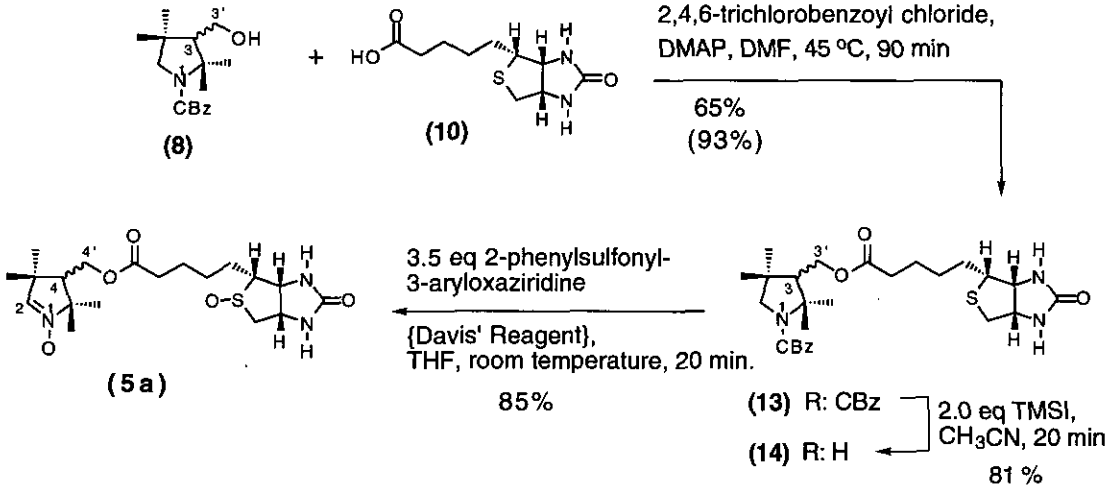




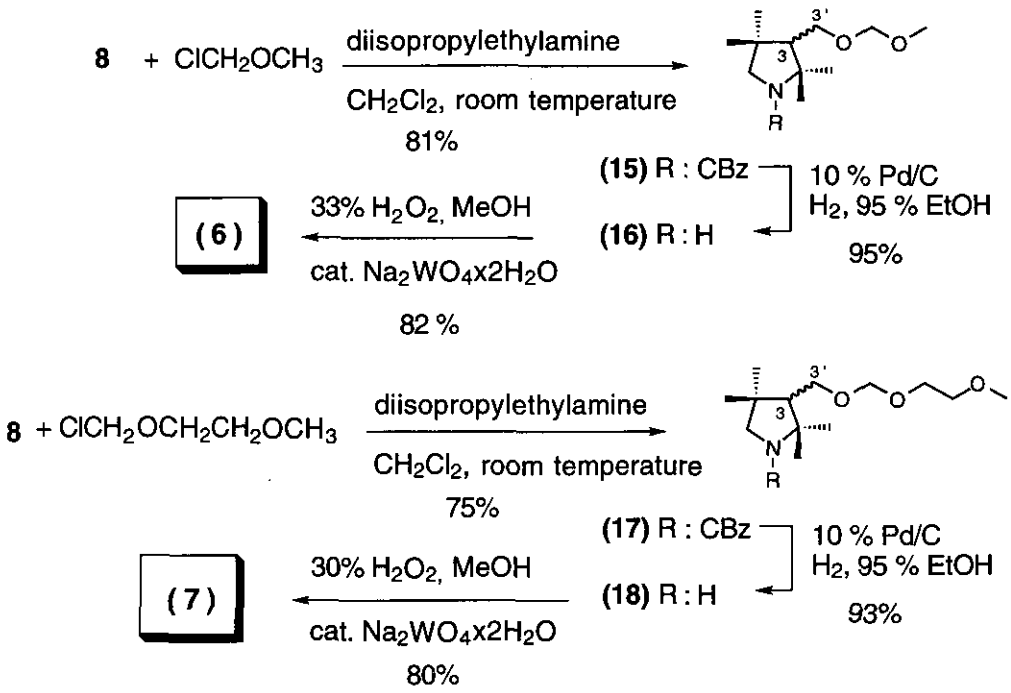
3-Hydroxymethyl-*N*-benzyloxycarbonyl-2,2,4,4-tetramethyl-1-pyrrolidine **(8)**, a common synthon required for the synthesis of spin traps **(4, 5, 6** and **7)** was synthesized as reported earlier.[8] Coupling of **8** with *N*-maleimidobutyric acid **(9)** was moderate (52%) when $SOCl_2$ (55 °C) was used for the activation of the -COOH group of **9**, whereas using Yamaguchi's[10] conditions of activation (2,4,6-trichlorobenzoyl chloride), the reaction was clean and high yielding. The product obtained was purified over silica gel using flash column chromatography (EtOAc, Hexanes; 1:3) to give **11**, CI-ms *m/z* 457 (M+1) in 93% yield. The *N*-protection was easily removed by controlled acidolysis. A brief exposure to 33% HBr in AcOH[11] gave **12**, CI-ms *m/z* 323 (M+1) in 85%. Oxidation by the routine method[12-14] did not succeed but using 2.2 equivalents of 2-phenylsulfonyl-3-aryloxaziridine (Davis' reagent)[15-16] gave spin trap **4**, CI-ms *m/z* 337 (M+1) in 87%. ^1H Nmr showed a peak at d 7.16 (*s*, 1H) for C_2-H, 6.75 (s, 2H) for olefinic protons of maleimido unit, 4.24 (d, J = 8.1 Hz, 2H) for C_4'-H. ^{13}C Nmr showed a peak at δ 172.0, 171.3 and 170.3 for three carbonyl units and 134.2 for C_2-carbon.



Activation of the -COOH group of biotin (Fluka, **10**) for coupling with synthon **(8)** using $SOCl_2$ or DCC-DMAP[17] failed to give the product **(13)**. Coupling reaction was clean when modified conditions for Yamaguchi's method[10] were used. The product was purified over silica gel with flash column chromatography (CH_2Cl_2, MeOH; 25:1). It was identified as **13** (65% or 93% based on the recovered starting material) from CI-ms *m/z* 518 (M+1), ^{1}H-nmr and ^{13}C-nmr (stereochemistry at C-3 was not determined). The deprotection of N-CBz group was resistant to catalytic hydrogenation (10% Pd-C), the hydrogen transfer (10% Pd-C) method, photochemical cleavage[18] and the use of 33% HBr-AcOH (15min) gave a complex mixture. However, 2.0 eq. of trimethylsilyl iodide (TMSI) in CH_3CN[19] for 20 min at room temperature gave the required deprotected product **(14)**, CI-ms *m/z* 384 (M+1) in 81% yield. As in the previous case, the oxidation was carried out using 3.5 equivalents of Davis' reagent to give the desired product **(5a)**, CI-ms *m/z* 398 (M+1-oxygen), in 85% yield. During the formation of spin trap **(5)** from the free amine derivative **(14)**, the sulfide group of biotin was also oxidized to sulfoxide.



Similarly, the alkylation of synthon **(8)** with the corresponding alkylating electrophiles, followed by the deprotection of N-CBz, and the oxidation gave nitrone derivatives **(6)** and **(7)** (see Experimental section).



EXPERIMENTAL

Dichloromethane and DMF were distilled over calcium hydride. THF was freshly distilled from sodium / benzophenone ketyl prior to use. ^{1}H Nmr and ^{13}C nmr spectra were recorded on Brucker-200. Unless specified, ^{1}H Nmr and ^{13}C nmr spectra were recorded in $CDCl_3$. Hewlett Packard Capillary Column Gas chromatograph, model-5890A and 5790 Series Mass Selective Detector were used. Chemical Ionization and FAB Mass spectra were obtained on Kratos Concept 2H. Flash column chromatography was performed as described by Still.[20]

N-Benzyloxycarbonyl-3-*N*-maleimidobutyryloxymethyl)-2,2,4,4-tetramethylpyrrolidine **(11):**

Procedure (a) Activation of carboxyl group of *N*-maleimidobutyric acid: A solution of *N*-maleimidobutyric acid (**9**, 1 mmol, 0.183 g) and $SOCl_2$ (2 mmol, 0.238 g) in benzene (15 ml) was warmed at 70 ^{o}C for 2 h. The solvent was evaporated *in vacuo* and the residue collected was used directly for the next step. Coupling: It was dissolved in THF (3 ml) and added dropwise to a solution of **8** (1 mmol, 0.291 g), Et_3N (1.5 mmol, 0.2 ml) and DMAP (2 mol %) in THF (15 ml) under argon. It was further stirred at 55 ^{o}C for 3 h. The reaction mixture was diluted with CH_2Cl_2 (50 ml) and quenched with NH_4Cl solution. The organic layer was separated, dried over and evaporated *in vacuo*. The residue was flash chromatographed (elution with EtOAc : Hexane, 1:3) to give **11** as an oil (0.237 g, 52 %). R_f 0.71 (EtOAc :

Hexane, 1:1). Anal. Calcd for $C_{25}H_{32}N_2O_6$: C, 65.78; H, 7.01; N, 6.14. Found: C, 65.80; H, 7.08; N, 6.17. ^{1}H Nmr 7.34 (br s, 5H, Ar-H), 6.69 (s, 2H, maleimido-H), 5.08 (s, 2H, -COOCH_2Ph), 4.18 (m, 2H, H-3'), 3.57 (t, J = 6.6 Hz, -OCOCH_2-), 3.43 and 3.12 (dd, J = 10.7, 10.7 Hz, H-5), 2.33 (t, J = 7.26 Hz, 2H, -NCH_2-), 1.95 (m, 3H, -OCOCH$_2$CH_2- and H-3), 1.48, 1.45, 1.09 and 1.01 (4s, 12H, 4xCH_3). ^{13}C Nmr 172.4, 170.7, 134.1, 128.5, 66.1, 62.0, 56.5, 36.9, 31.3, 28.7, 27.2, 23.7, 22.1 and 21.3.

Procedure (b), activation of carboxyl group of *N*-maleimidobutyric acid: To a solution of *N*-maleimidobutyric acid (**9**, 2.4 mmol, 0.439 g) and Et_3N (3 mmol, 0.4 ml) in THF (25 ml), under argon, added dropwise freshly prepared 2,4,6- trichlorobenzoyl chloride (2.4 mmol, 0.583 g) in THF (15 ml) at room temperature. The mixture was stirred for 1.5 h. Et_3N.HCl was filtered and the mother liquor was directly used for the coupling reaction. Coupling: It was added to a solution of **8** (2 mmol, 0.582 g), DMAP (2.2 mmol, 0.268 g) in THF (20 ml) under argon. The solution was stirred at room temperature for 30 minutes. The reaction mixture was diluted with CH_2Cl_2 (100 ml) and quenched with an addtion of pH 7 buffer solution (10 ml). The organic layer was collected, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was flash chromatographed (elution with EtOAc:Hexane, 1:3) to give **11** as an oil (0.848 g, 93%).

Deprotection of -N(CBz) group (**12**): 33% HBr in HOAc (1.0 ml) was added dropwise to a solution of **11** (1 mmol) in CH_2Cl_2 (5 ml) at room temperature. After 15 min, the reaction was quenched with 10% Na_2CO_3 solution. The organic layer was collected, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was flash chromatographed (elution with CH_2Cl_2 : MeOH, 40:3) to give **12** as an oil (0.276 g, 85%). R_f 0.25 (CH_2Cl_2 : MeOH, 10:1). Ms (CI ether) 323 (M+1, 87), 307 (4), 183 (21), 166 (50), 150 (14), 149 (100), 141 (140, 140 (100), 124 922), 111 (3) and 103 (7). HRms (M+1) $C_{17}H_{27}N_2O_4$, calcd 323.1963, found 323.1979. ^{1}H Nmr 6.68 (s, 2H, maleimido-H), 5.27 (s, 1H, NH), 4.07 (ABX, J = 6.4, 8.5, 11.4 Hz, 2H, H-3'), 3.51 (t, J = 6.7 Hz, 2H, -OCOCH_2-), 3.12 (br s, 2H, H-5), 2.27 (t, J = 7.2 Hz, 2H, -NCH_2-), 2.09 (t, J = 7.4 Hz, 1H, H-3), 1.85 (t, J = 6.9 Hz, 2H, -OCOCH$_2$CH_2-), 1.65, 1.47, 1.25 & 1.12 (4s, 12H, 4xCH_3). ^{13}C Nmr 171.7, 170.0, 133.8, 66.5, 60.4, 54.6, 39.6, 36.4, 30.7, 28.7, 28.1, 23.2, 22.7 and 21.2.

Oxidation procedure (**4**): Freshly prepared Davis' reagent (2.2 mmol, 0.574 g) was added to a solution of **12** (1 mmol, 0.322 g) in THF (15 ml). It was stirred at room temperature for 20 min. The residue obtained was filtered and the solvent was collected and evaporated *in vacuo*. The residue was flash chromatographed (elution with CH_2Cl_2 : MeOH, 40:1.5) to give **4** as an oil (0.292 g, 87%). R_f 0.66 (CH_2Cl_2 : MeOH, 10:1). Ms (CI, ether) 337 (M+1, 1), 323 (10), 322 (17), 321 (88, M+1-oxygen), 232 (21), 186

(2), 158 (5), 150 (14), 149 (100), 139 (4), 138 (34), 124 (12), 122 (4), 111(4), 105 (1) and 103 (7). HRms (M+1-oxygen) $C_{17}H_{25}N_2O_4$, calcd 321.182, found 321.1832. ^{1}H Nmr 7.16 (s, 1H, H-2), 6.75 (s, 2H, maleimido-H), 4.24 (d, J = 8.1 Hz, 2H, H-3'), 3.63 (t, J = 6.8 Hz, 2H, -OCOCH$_2$-), 2.39 (t, J = 7.4 Hz, 2H, -NCH$_2$-), 1.97 (m, 3H, -OCOCH$_2$CH$_2$- and H-3), 1.40, 1.25, 1.15 and 1.08 (4s, 12H, 4xCH$_3$). ^{13}C Nmr 172.0, 171.3, 170.3, 134.2, 74.7, 62.4, 53.2, 51.6, 37.0, 41.4, 31.2, 27.5, 24.3, 23.8 and 20.7.

Coupling of **8** with biotin to give **14**, activation of the carboxyl group of biotin: To a solution of biotin (**10**, 2 mmol, 0.488 g) and Et_3N (3 mmol, 0.4 ml) in DMF (15ml), under argon was added dropwise freshly prepared 2,4,6- trichlorobenzoyl chloride (2 mmol, 0.486 g) in DMF (5 ml) at 45 °C. The mixture was stirred for 1.5h. It was directly used for the coupling step. *Coupling:* To the above solution, added **8** (0.582 g, 2 mmol), DMAP (3 mmol, 0.366 g) under argon. The mixture was stirred at 45 °C for 1.5 h. The reaction was diluted with CH_2Cl_2 (100 ml) and quenched with pH 7 buffer solution (20 ml). The organic layer was collected, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was flash chromatographed (elution with CH_2Cl_2 : MeOH, 30:1) to give **13** as an oil (0.672 g, 65% and 93% based on the recovered starting material). Rf 0.47 (CH_2Cl_2 : MeOH, 10:1). Ms (CI, ether) 518 (M+1, 3), 517 (8), 384 (9), 382 (3), 292 (7), 227 (8), 150 (11), 140 (4), 133 (4), 124 (3) and 103 (8). Anal. Calcd for $C_{27}H_{39}N_3O_5S_1$: C, 62.66; H, 7.54; N, 8.12. Found: C, 62.70 ; H, 7.60; N, 8.14. ^{1}H Nmr 7.36 (br s, 5H), 6.43 (br s, 1H), 6.12 (br s, 1H), 5.10 (s, 2H), 4.14-4.60 (m, 4H), 3.45 (d, J = 11 Hz, 1H), 3.15 (m, 2H), 2.91 (m, 1H), 2.74 (d, J = 12.6 Hz, 1H), 2.37 (t, J = 7.4 Hz, 2H), 2.04 (m, 1H), 1.70 (m, 6H) and 1.51, 1.47, 1.11, 1.03 (4s, 12H). ^{13}C Nmr 170.3, 160.9, 153.5, 136.8, 65.8, 61.8, 61.6, 59.9, 56.3, 55.3, 40.3, 37.4, 33.8, 28.5, 28.2, 28.0, 26.9, 24.5, 21.9 and 21.0 (aromatic carbons are excluded).

Deprotection of -N(CBz) group (**14**): Trimethylsilyl iodide (2 mmol, 0.4 g) was added dropwise to a solution of **13** (1 mmol, 0.517 g) in CH_3CN (25 ml) at room temperature. After 20 min, the reaction was quenched with 10% Na_2CO_3 solution. The organic layer was collected, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was flash chromatographed (elution with CH_2Cl_2 : MeOH, 10:1) to give **14** as an oil (0.31 g, 81%). Rf 0.42 (CH_2Cl_2: MeOH, 5:1). Anal. Calcd for $C_{19}H_{33}N_3O_3S_1$: C, 59.53; H, 8.61; N, 10.96. Found: C, 59.61; H, 8.65; N, 10.98. Ms (CI, ether) 384 (M+1, 6), 383 (2), 328 (1), 270 (1), 242 (2), 227 (5), 225 (3), 209 94), 197 94), 158 (30), 156 (9), 149 (100), 140 (11), 129 (16), 119 (6), 117 (7), 113 (6), 105 (3), 103 (19) and 101 (3). ^{1}H Nmr (CD_3OD) 4.28 (ABX, J = 4.6, 7.2, 8.0 Hz, 2H), 4.12 (ABX, J = 6.4, 8.4, 11.4 Hz, 2H), 3.10 (m, 1H), 3.04 (d, J = 0.9 Hz, 2H), 2.84 (dd, J = 4.7, 9.2 Hz, 1H), 2.60 (d, J = 12.7 Hz, 1H),

2.29 (t, J = 7.1 Hz, 2H), 2.04 (dd, J = 6.5, 6.4 Hz, 1H), 1.55 (m, 6H), 1.44, 1.33, 1.16 and 1.02 (4s, 12H). ^{13}C Nmr (CD_3OD) 174.7, 166.0, 67.2, 63.4, 61.9, 61.6, 57.0, 56.5, 41.8, 41.0, 34.7, 29.7, 29.3, 28.4, 25.8 and 22.4.

Oxidation procedure (**5a**): Freshly prepared Davis' reagent (3.5 mmol, 0.9135 g) was added to a solution of **14** (1 mmol, 0.383 g) in THF (15 ml). It was stirred at room temperature for 20 min. The residue obtained was filtered and the solvent was collected and evaporated *in vacuo*. The residue was flash chromatographed (elution with CH_2Cl_2 : MeOH, 10:1) to give **5a** as an oil (0.351 g, 85 %). R_f 0.25 (CH_2Cl_2 : MeOH, 5:1). Anal. Calcd for $C_{19}H_{31}N_3O_5S_1$: C, 55.20; H, 7.50; N, 10.16. Found: C, 55.50; H, 7.70; N, 10.26 . Ms (CI, ether) 399 (M+2-oxygen, 23), 398 (M+1-oxygen, 100), 382 (16), 376 (2), 321 (1), 243 (3), 229 (1), 227 (2), 199 (3), 184 (3), 171 (3), 159 (2), 158 (21), 156 (18), 123 (4) and 111 (2). Ms (FAB) 399 (M+2-oxygen, 31), 398 (M+1-oxygen, 100), 384 (14), 382 (77), 380 (11), 351 (21), 348 (5), 327 (5), 315 (9), 299 (6), 284 (9), 245 (11), 243 (15), 227 (14), 223 (80), 210 (14), 207 (14), 203 (13), 199 (11), 197 (11), 194 (10), 191 (11), 185 (25), 171 (14), 156 (15), 152 (11), 140 (230, 139 (17), 138 (68), 137 (26), 136 (25), 135 (11) and 133 (12). ^1H Nmr (CD_3OD) 4.52 (ABX, J = 4.5, 5.1, 7.8 Hz, 2H), 4.14 (d, J = 8.0 Hz, 2H), 3.42 (dd, J = 1.8, 13.2 Hz, 1H), 3.02 (m, 2H), 2.32 (t, J = 7.0 Hz, 2H), 1.80 (m, 3H), 1.50 (m, 4H), 1.26, 1.15, 1.03, 0.98 (4s, 12H). ^{13}C Nmr (CD_3OD) 174.9, 167.4, 71.9, 63.1, 59.4, 58.4, 55.7, 54.4, 52.7, 50.3, 34.6, 31.5, 28.1, 26.6, 25.8, 24.6 and 20.7.

(15): To a solution of **8** (1.45 g, 5.0 mmol) in dichloromethane (50 ml) was added diisopropylethylamine (1.29 g, 10 mmol) dropwise at 25 °C. It was followed by a dropwise addition of a solution of methoxymethyl chloride (0.55 g, 6.0 mmol) in dichloromethane (10 ml). After stirring at 25 °C for 2 h, it was diluted with dichloromethane (200 ml) and buffer solution (pH 7, 25 ml). The organic layer was collected, dried over $MgSO_4$ and evaporated. The resulting solid residue was purified by flash chromatography over silica gel and eluted with 1:5 ethylacetate-hexane to give **15** as an oil (1.35 g, 81 %). R_f 0.19 (EtOAc: hexane, 1:5). Anal. Calcd for $C_{19}H_{29}N_1O_4$: C, 68.05; H, 8.65; N, 4.17. Found: C, 68.35; H, 8.68; N, 4.19. ^1H Nmr 7.34 (br s, 5H, Ar-H), 5.10 (s, 2H, -OCH_2O-), 4.60 (s, 2H, -OCH_2Ph), 3.58 (d, J = 7.2 Hz, C_2-H), 3.40 (m, 2H, H-4'), 3.36 (s, -OCH_3, 3H), 1.94 (d, J = 7.2 Hz, H-4), 1.47, 1.43, 1.07 and 0.97 (4s, 12H, 4x-CH_3). ^{13}C Nmr 152.5, 96.5, 66.6, 64.9, 60.0, 57.3, 55.2, 37.3, 28.5, 27.1, 21.9 and 20.9.

Deprotection of -N(CBz) group (**16**): A solution of **15** (10.0 mmol, 3.35 g) in 95% ethanol (50 ml) and 10 % palladium on charcaol (200 mg) was hydrogenated under atmospheric pressure for 10 h. The mixture was filtered over florisil (5 g) and the solvent was evaporated to give **16** as an oil (1.90 g, 95 %). Anal. Calcd for $C_{11}H_{23}N_1O_2$: C, 65.67; H, 11.44; N, 6.96. Found: C, 65.78; H, 11.54; N, 6.98 . Ms (%) 45 (64),

71 (100), 96 (10), 111(17), 140 (92), 186 (58), 201(M+, 2). ^{13}C Nmr 96.1, 65.7, 61.0, 59.0, 57.4, 56.4, 54.7, 41.6, 30.9, 28.8, 23.2 and 21.9.

Oxidation procedure (**6**): To a stirred solution of **16** (5.0 mmol, 1.0 g) and $Na_2WO_4x2H_2O$ (5.0 %, 0.016g) in methanol (25 ml) was added dropwise 33 % hydrogen peroxide (15.0 mmol, 0.56 ml) at O °C. After stirring at 0 °C for 4 h, the solvent was evaporated to dryness. The solid residue was taken up with dichloromethane (50 ml), washed with brine (10 ml), dried over and evaporated to dryness. The residue was purified by flash chromatography over silica gel and eluted with 20:1 dichloromethane: methanol to give **6** *(0.88 g, 82 %) as a yellow oil. Rf 0.5 (CH_2Cl_2 : MeOH, 10:1).* Anal. Calcd *for* $C_{11}H_{21}N_1O_3$: C, 61.39; H, 9.76; N, 6.51. Found: C, 61.45; H, 9.80; N, 6.60. Ms 45(100), 69(19), 86(15), 124(11), 138(14), 170(8), 200(5), 215(M+, 5). ^{1}H Nmr 6.5 (s, 1H, H-2), 4.47 (s, 2H, -OCH_2O-), 3.45 (m, 2H, -CH_2OCH_2-), 3.23 (s, 3H, -OCH_3), 2.11 (t, J = 7.6 Hz, H-4), 1.31, 1.21, 1.1 and 0.97 (4s, 12H, 4x-CH_3). ^{13}C Nmr 140.2, 96.4, 75.6, 64.0, 55.2, 52.8, 39.9, 28.0, 27.3, 21.2 and 20.9.

(17): To a solution of **8** (1.45 g, 5.0 mmol) in dichloromethane (50 ml) was added diisopropylethylamine (1.29 g, 10 mmol) dropwise at 25 °C. It was followed by a dropwise solution solution of methoxyethoxymethyl chloride (0.74g, 6.0 mmol) in dichloromethane (10 ml). After stirring at 25 °C for 32 h, it was diluted with dichloromethane (200 ml) and pH 7 buffer solution (25 ml). The organic layer was collected, dried over $MgSO_4$ and evaporated. The resulting residue was purified by flash chromatography over silica gel and eluted with 1:5 ethylacetate-hexane to give **17** as an oil (1.42g, 75 %). Rf 0.19 (EtOAc: hexane, 1:5).Anal. Calcd for $C_{21}H_{33}N_1O_5$: *C, 66.49; H, 8.70; N, 3.69. Found: C, 66.72;* H, 8.90; N, 3.72. ^{1}H Nmr 7.39 (br s, 5H, Ar-H), 5.06 (s, 2H, -OCH_2Ph), 4.69 (s, 2H, -OCHO-), 3.65 (m, 4H, -OCH_2CH_2O-), 3.37 (s, 3H, -OCH_3), 3.10 (d, 1H, J 7.2Hz, H-4), 1.93 (t, J 7.2 Hz, H-3), 1.93, 1.42, 1.06 and 0.96 (4s, 12H, 4x-CH_3). ^{13}C Nmr 153.3, 128.0, 95.3, 71.3, 66.6, 65.5, 64.8, 62.7, 59.8, 57.1, 37.2, 28.4, 26.9, 21.7 and 20.8,

Deprotection of -N(CBz) group (**18**): A solution of **17** (10.0 mmol, 3.79 g) in 95% ethanol (50 ml) and 10 % palladium on charcaol (379 mg) was hydrogenated under atmospheric pressure for 10 h. The mixture was filtered over florisil (5 g) and the solvent was evaporated to give of **18** as an oil (2.27 g, 93 %). Anal. Calcd *for* $C_{13}H_{27}N_1O_3$: *C, 63.67; H, 11.02; N, 5.71. Found: C, 63.88;* H, 11.10; N, 5.78 . Ms 59(38), 71(77), 111(17), 124(72), 140(100), 141(10), 154(4), 230(38), 245(M+,1). ^{13}C Nmr 95.0, 71.1, 66.2, 65.7, 60.7, 59.1, 58.2, 56.9, 41.6, 30.9, 28.8, 23.3 and 21.8.

Oxidation procedure (**7**): To a stirred solution of **18** (5.0 mmol, 1.22 g) and $Na_2WO_4x2H_2O$ (5.0 %, 0.016g) in methanol (25 ml) was added dropwise 33 % hydrogen peroxide (15.0 mmol, 0.56 ml) at O °C. After stirring at 0 °C for 4 h, the solvent was evaporated to dryness. The solid residue was taken up with dichloromethane (50 ml), washed with brine (10 ml), dried over and evaporated to dryness. The residue was purified by flash chromatography over silica gel and eluted with 20:1 dichloromethane : methanol to give **7** as an oil (1.03g, 80 %). R_f 0.45 (CH_2Cl_2 : MeOH, 10:1). Anal.Calcd for $C_{13}H_{25}N_1O_4$: C, 60.23; H, 9.26; N, 5.40. Found: C, 60.45; H, 9.45; N, 5.52 . ^{1}H Nmr 6.6 (s, 1H, H-2), 4.5 (s, 2H,-OCH_2O-), 3.51 (m, 4H, -OCH_2CH_2O-), 3.39 (m, 2H, H-4'), 3.2 (s, 3H, -OCH_3), 2.1 (d, J = 7.6 Hz, 1H, H-4), 1.29, 1.18, 1.09 and 0.96 (4s, 12H, 4x-CH_3). Ms 55(20), 59(100), 69(27), 89(76), 138(9), 140(5), 154(40. 170(20), 200(8), 259(1). ^{13}C Nmr 95.0, 71.1, 66.2, 65.7, 60.7, 59.1, 58.2, 56.9, 41.6, 30.9, 28.8, 23.3 and 21.8.

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