Synthesis of 2-Substituted Pyrrolidine Nitroxide Radicals

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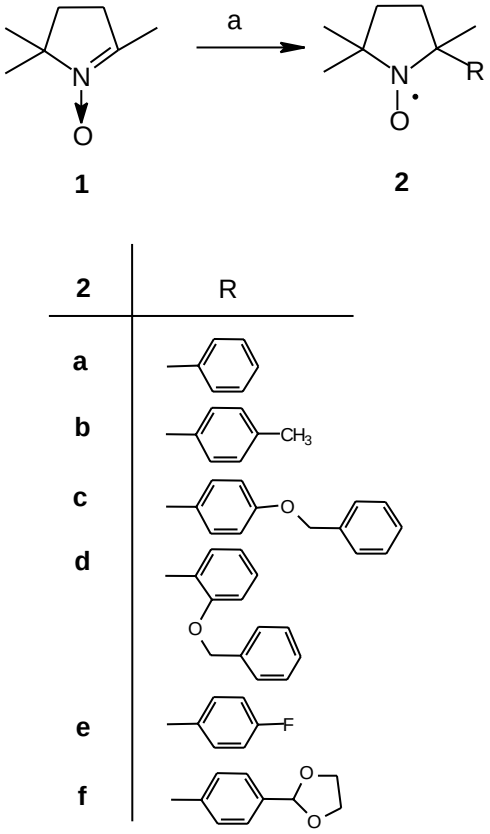
Dedicated to Prof. Douglas Lloyd on the occasion of his 80th birthday

Abstract: Grignard reaction of nitrone **1** with arylmagnesium bromides afforded different pyrrolidine nitroxide radicals **2a−f**, which were converted further to spin labelled ebselen **6**, saccharine **11,** phenols **5, 12, 13,** quinone **16** and heterocycles **20, 21, 24.** Paramagnetic sulfonyl chlorides **7a, 7b, 9a** and methanethiosulfonate **25d** capable of amino and thiol labelling, respectively, were also prepared.

Key words: Grignard reaction, free radicals, heterocycles, phenols, quinones, selenium, oxidations, reductions

Nitroxides have attracted much attention of the scientific community as spin labels,[1] SOD mimics,[2] potential MRI agents,[3] building blocks for organic magnets,[4] and spin traps of carbon centered radicals.[5] Accordingly, several synthetic methods have been developed: oxidation of triacetonamine,[6] Grignard reaction of nitrones,[7−12] Grignard reaction of phthalimide followed by oxidation,[13] and Binger reaction of fullerene followed by oxidation.[14] In this paper we describe the Grignard reaction of 2,2,5-trimethyl-3,4-dihydro-2*H*-pyrrol-1-oxide (**1**)[15] with aromatic Grignard reagents followed by subsequent functionalization leading to paramagnetic heterocycles and biologically active compounds which can not be obtained directly by Grignard reactions of nitrone **1**. Nitrone **1** was reacted with phenylmagnesium bromide,[8] 4-methylphenylmagnesium bromide, 4-benzyloxyphenylmagnesium bromide,[16] 2-benzyloxyphenylmagnesium bromide,[17] 4-fluorophenylmagnesium bromide[9] and 4-[(1,3)-dioxolane-2-yl]phenylmagnesium bromide[18] in diethyl ether to give the corresponding nitroxides **2a−f** after workup and oxidation with MnO_2/O_2 (Scheme 1).



Reagents and conditions: (a) RMgX (1.66 equiv), Et_2O, 0 °C → 35 °C, 1 h, r.t., 12 h, then aq NH_4Cl, MnO_2 (cat.)/O_2, (44−89%)

Scheme 1

Nitration of 2,5,5-trimethyl-2-phenylpyrrolidin-1-yloxyl radical (**2a**) gave 2,5,5-trimethyl-2-(3-nitrophenyl)pyrrolidine-1-yloxyl radical[8] which was reduced with ammonium formate in the presence of Pd/C catalyst[19] to the paramagnetic arylamine **4**. This compound was diazotized with HNO_2 and the diazonium compound was decomposed to 2,5,5-trimethyl-2-(3-hydroxyphenyl)pyrrolidine-1-yloxyl radical (**5**). Reaction of compound **4** with 2-(chloroseleno)benzoyl chloride[20] in the presence of triethylamine in dichloromethane yielded the spin labelled derivative of antioxidant[21] ebselen (**6**)**,** which also exhibits glutathion peroxidase,[22] and peroxynitrite scavenging activity.[23] Chlorosulfonation of compound **2a** could be accomplished without irreversible destruction of *N*-oxyl group and gave a 2:1 mixture of 2,5,5-trimethyl-2-(3-chlorosulfonylphenyl)pyrrolidine-1-yloxyl radical (**7a**) and 2,5,5-trimethyl-2-(4-chlorosulfonylphenyl)pyrrolidine-1-yloxyl radical (**7b**). The formation and ratio of isomers were evaluated by ^{1}H NMR after the reaction of chlorosulfonyl derivative with glycine methyl ester yielding a 2:1 mixture of [3-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzenesulfonamido]glycine methyl ester radical (**8a**) and [4-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzenesulfonamido]glycine methyl ester radical (**8b**), which were investigated by ^{1}H NMR (Scheme 2). As far as we know this is the first example of direct chlorosulfonation in the presence of a free radical. Fluorosulfonyl compounds were synthesized earlier by covalent coupling of

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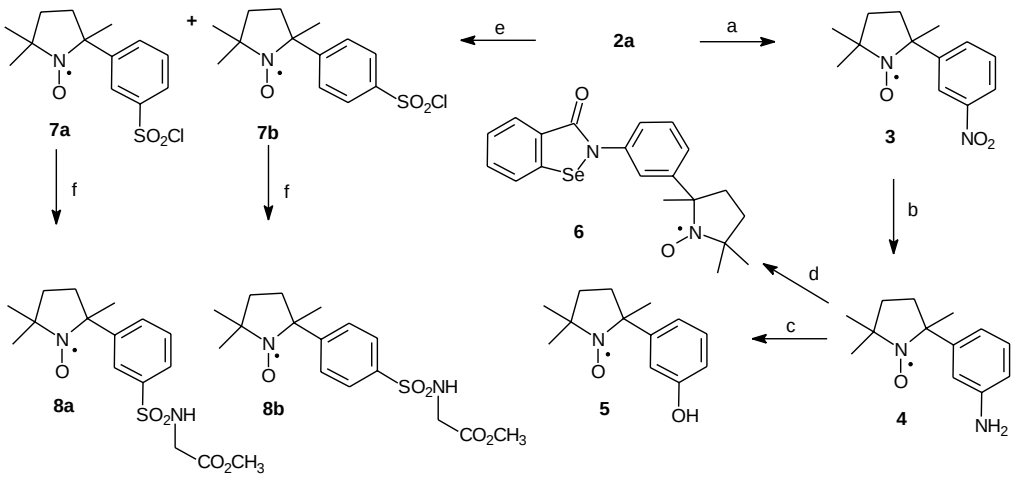
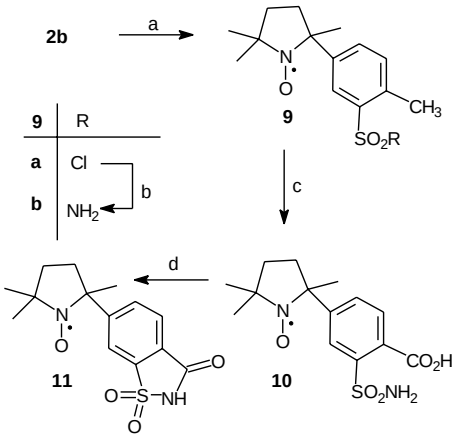


Reagents and conditions: (a) 98% H_2SO_4/67% HNO_3, 0 °C → r.t., 30 min., then 40% aq NaOH, (60%); (b) HCO_2NH_4 (6.0 equiv)/Pd-C (cat.), 40 °C, 2 h, then PbO_2 (cat.), O_2, (58%); (c) $NaNO_2$ (1.0 equiv)/2 M aq HCl, H_2O, 0 °C → 40 °C, $NaHCO_3$/PbO_2 (cat.)/O_2, (27%); (d) 2-(chloroseleno)benzoyl chloride (1.0 equiv)/CH_2Cl_2/Et_3N (2.2 equiv), r.t., 45 min., (55%); (e) $ClSO_3H$, 0 °C → r.t., 2 h, $NaHCO_3$, (73%); (f) $H_2NCH_2CO_2Me$ (1.0 equiv), $NaHCO_3$ (2 equiv), H_2O/dioxane, r.t., 2 h, (72%)

Scheme 2

nitroxide and fluorosulfonyl starting compounds and successfully applied for labelling of active sites of trypsin and α-chymotrypsin.[24] Sulfonation of a nitroxide amine precursor was reported very recently.[25]

To avoid the formation of isomers, compound **2b** containing a methyl group in *para* position was chlorosulfonated to afford the 2,5,5-trimethyl-2-(3-chlorosulfonyl-4-methylphenyl)pyrrolidine-1-yloxyl radical (**9a**) which was converted to the corresponding sulfonamide **9b** with aqueous NH_3 solution. Compound **9b** was oxidized in 2% aqueous NaOH solution with $KMnO_4$ to the corresponding acid **10**, which was cyclized to spin labelled saccharine[26] **11** on heating (Scheme 3).



Reagents and conditions: (a) $ClSO_3H$, 0 °C → r.t., 2 h, $NaHCO_3$, (63%); (b) 27% aq NH_4OH, 100 °C, r. t., 12 h, (75%); (c) 2% aq NaOH/$KMnO_4$ (1.75 eqiuv), 35 °C → r. t., 24 h, oxalic acid, then 5% aq H_2SO_4, (35%); (d) r.t. → 200 °C → r.t.

Scheme 3

To prepare the *para*- and *ortho*-substituted phenols beside the *meta*-substituted phenol **5,** the corresponding 4-benzyloxy derivative **2c** and 2-benzyloxy derivative **2d** were subjected to transfer hydrogenolysis to give the deprotected 2,5,5-trimethyl-2-(4-hydroxyphenyl)pyrrolidine-1-yloxyl radical (**12**) and 2,5,5-trimethyl-2-(2-hydroxyphenyl)pyrrolidine-1-yloxyl radical (**13**). Treatment of compound **13** with nitrated silica gel[27] in dichloromethane yielded the 2,5,5-trimethyl-2-(2-hydroxyphenyl-3-nitro)pyrrolidine-1-yloxyl radical (**14**) and 2,5,5-trimethyl-2-(2-hydroxyphenyl-5-nitro)pyrrolidine-1-yloxyl radical (**15**) in a 1:1 ratio. Compound **15** was reduced to the light sensitive spin labelled 4-aminophenol derivative, which was oxidized directly with aqueous $AgNO_3$ solution[28] to 2-(1-oxyl-2,2,5-trimethyl-pyrrolidine-2-yl)[1,4]benzoquinone (**16**) (Scheme 4). Spin labelled quinones reported earlier from our laboratory[29] were synthesized to mimic menadione (vitamin K_3). Compound **16** is expected to act as a SH specific reagent[30] and as an oxidation/reduction sensitive compound, while the oxidation state corresponding to this is indicated in its EPR spectrum.

Synthesis of five-membered heterocycles annulated to benzene paramagnetic compounds were reported earlier.[9] We extended this procedure for the synthesis of six- and seven-membered heterocycles annulated to benzene compounds. Compound **2e** was nitrated to give *o*-nitrofluoro derivative **17** and the fluorine atom was replaced by aromatic nucleophilic substitution with benzylamine to provide the 2,5,5-trimethyl-2-(4-benzylamino-3-nitrophenyl)pyrrolidine-1-yloxyl radical (**18**). Simultaneous reduction of nitro group and debenzylation of amine afforded the paramagnetic *o*-phenylene diamine **19,** which was reacted with benzil to give the 2-(2,3-diphenylquinoxalin-6-yl)-2,5,5-trimethylpyrrolidin-1-yloxyl radical (**20**), and with penta-2,4-dione in NaOAc/AcOH buffer[31]

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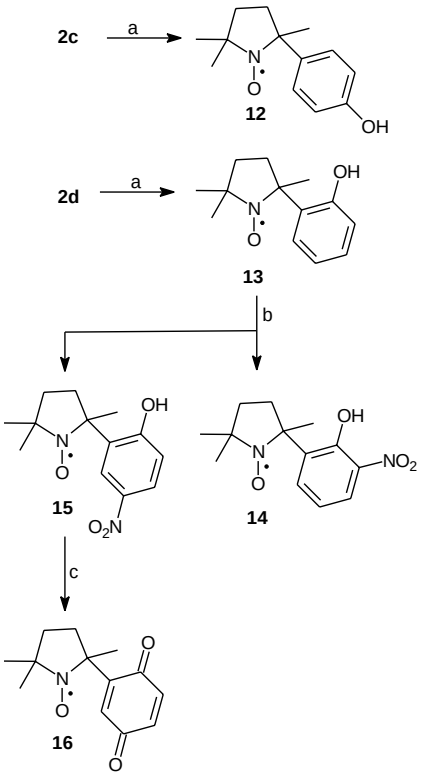


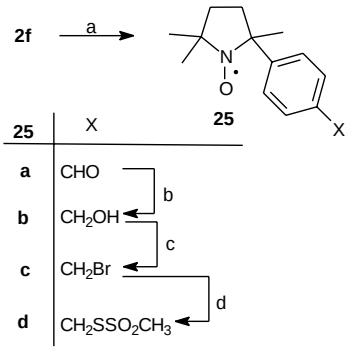
Reagents and conditions: (a) HCO_2NH_4 (15 equiv)/Pd-C (cat.)/MeOH, 3 h, 65 °C, then PbO_2 (cat.)/O_2 (12−32%); (b) $SiO_2.HNO_3$ (1.1 equiv), CH_2Cl_2, 10 °C → r.t., 45 min, (23−24%); (c) HCO_2NH_4 (6 equiv)/Pd-C (cat.)/MeOH, 40 °C, 2 h, then PbO_2 (cat.)/O_2; (c) 0.1 M aq $AgNO_3$ (1.5 equiv), then $NaHCO_3$, MnO_2 (5.0 equiv)/O_2

Scheme 4

to afford the 2-(2,4-dimethyl-3*H*-benzo[*b*][1,4]diazepin-7-yl)-2,5,5-trimethylpyrrolidin-1-yloxyl radical (**21**).

Earlier we have prepared the 2,5,5-trimethyl-2-(4-cyano-3-nitrophenyl)pyrrolidine-1-yloxyl radical[9] (**22**) from compound **17** with some modification using the less hazardous tetraethylammonium cyanide instead of KCN in acetonitrile. Compound **22** was oxidized to 2,5,5-trimethyl-2-(4-formamido-3-nitrophenyl)pyrrolidine-1-yloxyl radical with H_2O_2 in the presence of K_2CO_3 and reduced to spin labelled anthranilic amide **23** with ammonium formate catalyzed by Pd/C. Diazotization of compound **23** yielded 7-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)-3*H*-benzo[*d*][1,2,3]triazin-4-one radical (**24**) (Scheme 5).

The 2,5,5-trimethyl-2-(3-methanethiosulfonylmethylprop-1-ynyl)pyrrolidin-1-yloxyl radical and 2,5,5-trimethyl-2-(3-methanesulfonylmethylprop-1-enyl)pyrrolidin-1-yloxyl radicals have been described earlier as thiol-specific spin labels from our laboratory.[10] To obtain a close aromatic analog, 2,5,5-trimethyl-2-(4-methanesulfonylmethylphenyl)pyrrolidine-1-yloxyl radical (**25d**) was prepared starting from protected formyl compound **2f**. The deprotected aldehyde 2,5,5-trimethyl-2-(4-formyl)pyrrolidine-1-yloxyl radical (**25a**) was prepared by treating **2f** with 5% aqueous HCl/THF.[32] Aldehyde **25a** was reduced to the alcohol **25b** with $NaBH_4$, then converted to the corresponding mesylate which without isolation, was substituted by bromine by means of LiBr in acetone to give **25c**.[33] Compound **25c** was converted to **25d** containing the thiol-specific label. This offers a newly substituted label as compared to the previously successfully applied 3-substituted labels[34] (Scheme 6).



Reagents and conditions: (a) 2 M HCl/THF, 6 h, r.t. (70%); (b) $NaBH_4$ (2.0 eqiuv), EtOH, r.t., 30 min, (82 %); (c) MsCl (1.2 equiv)/Et_3N (1.2 eqiv), CH_2Cl_2, 0 °C → r.t., then LiBr (1.4 equiv)/acetone, 56 °C, 30 min, (48%); (d) $NaSSO_2CH_3$ (2.0 eqiuv)/EtOH/H_2O, 78 °C, 15 min, (30%)

Scheme 6

In conclusion, 2-substituted pyrroline nitroxides available from nitrone **1** can be varied partly by choosing the proper Grignard reaction and by application of further functionalization procedures. This method allowed synthesis of spin labelled ebselen, quinone, saccharine and other heterocycles as well as amino- and thiol-specific spin labels.

Melting points were determined with a Boetius micro melting point apparatus and are uncorrected. Elemental analyses (C, H, N, S) were performed on Fisons EA 1110 CHNS elemental analyzer. The IR (Specord 75) spectra were in each case consistent with the assigned structure. Mass spectra were recorded on a VG TRIO-2 instrument in the EI mode (70 eV, direct inlet) or with thermospray technique. Samples were analyzed in the by-pass mode. 10 µLof the sample solution in MeOH was introduced via the thermospray interface. The mobile phase was MeOH/H_2O (1:1) containing 0.1 M NH_4OAc. The capillary tip temperature was 230 °C, the electrode voltage 180 V and the source temperature 210 °C. The ESR spectra were obtained from 10^{-5} molar solution ($CHCl_3$), using Bruker ECS-106 spectrometer. All monoradicals exhibit three equidistant lines with a_N = 14.3−14.8 G. ^1H NMR spectra were recorded with a Varian INOVA 400 WB spectrometer at 400 MHz at 25 °C. Chemical shifts are given in ppm, relative to TMS as external standard. To obtain high resolution NMR spectra of the radicals they were reduced with excess codissolved $(PhNH)_2$. Flash column chromatography was performed on Merck Kieselgel 60 (0.040−0.063 mm). Qualitative TLC was carried out on commercially prepared plates (20 × 20 × 0.02 cm) coated with Merck Kieselgel GF_{254}. Compounds **1**,[15] **2a**,[8] **2e**,[9] **3**,[8] **17**,[9] **19**,[9] **22**,[9] and 2-(chloroseleno)benzoyl chloride[20] were prepared according to published procedures. The physical and spectral data of all new compounds are listed in the Table.

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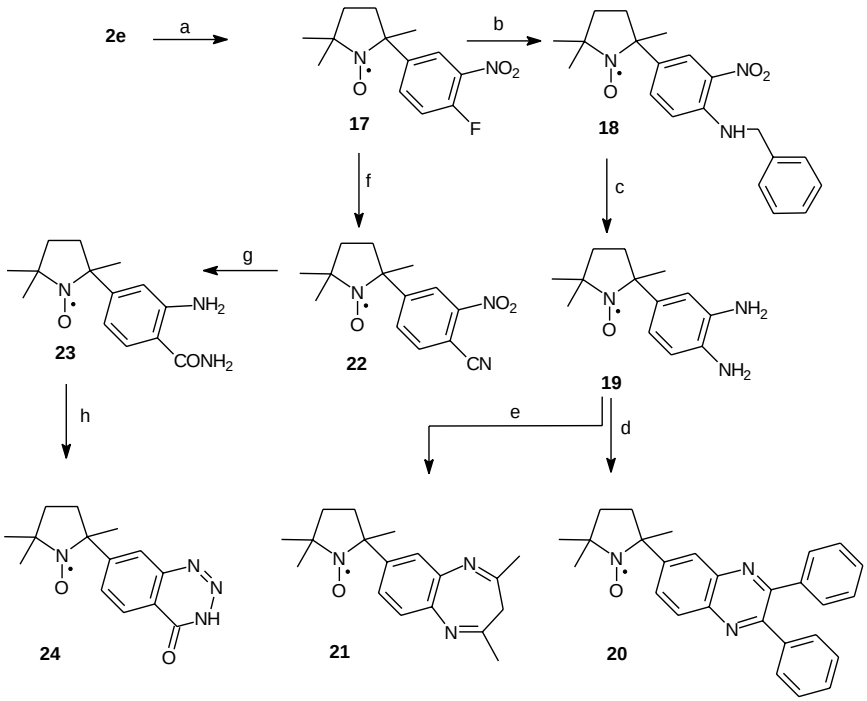

Reagents and conditions: (a) 98% H_2SO_4/67% HNO_3, 0 °C → r.t., 30 min, then K_2CO_3, (72%); (b) $PhCH_2NH_2$ (2.0 equiv)/THF, 65 °C, 1 h, (40 %); (c) (a) HCO_2NH_4 (15 equiv)/Pd-C (cat.)/MeOH, 3 h, 65 °C, then PbO_2 (cat.)/ O_2 (25 %); (d) benzil (1.0 eqiv)/EtOH, 78 °C, 1 h (27%); (e) $MeCOCH_2COMe$ (1 equiv)/aq AcOH/NaOAc (pH 4.8), 48 h, r.t. (31%), (f) Et_4NCN (1.0 eqiv)/MeCN, 50 °C, 2 h (41%); (g) HCO_2NH_4 (6 equiv)/Pd-C (cat.)/MeOH, 40 °C, 2 h, then PbO_2 (cat.)/O_2 (39%); (h) $NaNO_2$ (1.0 equiv)/2 M aq HCl, 0 °C, 10 min, 0°C → r.t., (47%)

Scheme 5

Nitroxides 2b–f by Grignard Reaction; General Procedure

To a stirred solution of the freshly prepared Grignard reagent (50.0 mmol) in anhyd Et_2O (50 mL) was slowly added a solution of the nitrone **1** (3.81 g, 30.0 mmol) in anhyd THF (15 mL) under N_2 at 0 °C and the mixture was further stirred and refluxed for 1 h. The mixture was allowed to stay at r.t. for overnight, then quenched with sat aq NH_4Cl solution (30 mL). The organic phase was separated, washed with brine (30 mL) and evaporated to dryness. The residue was dissolved in $CHCl_3$ (30 mL), dried ($MgSO_4$), MnO_2 (174 mg, 2.0 mmol) was added and O_2 was bubbled through the mixture for 30 min. The orange solution was filtered, evaporated and purified by flash column chromatography (hexane/Et_2O).

2,5,5-Trimethyl-2-(4-methylhenyl)pyrrolidin-1-yloxyl Radical (2b)

Yield: 4.00 g (61%); red oil; R_f 0.37 (hexane/Et_2O, 2:1).

2,5,5-Trimethyl-2-(2-benzyloxyphenyl)pyrrolidin-1-yloxyl Radical (2c)

Yield: 8.30 g (89%); yellow solid; mp 74–75 °C; R_f 0.16 (hexane/Et_2O, 2:1).

2,5,5-Trimethyl-2-(4-benzyloxyphenyl)pyrrolidin-1-yloxyl Radical (2d)

Yield: 4.15 g (44%); yellow solid; mp 90–91 °C; R_f 0.27 (hexane/Et_2O, 2:1).

2-[4-(1,3)-Dioxolan-2-yl-phenyl]-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (2f)

Yield: 3.9 g (47%); orange oil; R_f 0.29 (hexane/EtOAc, 2:1).

2,5,5-Trimethyl-2-(3-aminophenyl)pyrrolidin-1-yloxyl Radical (4)

To a stirred solution of compound **3** (4.98 g, 20.0 mmol) and HCO_2NH_4 (7.56 g, 0.12 mol) in MeOH (40 mL) was added Pd/C (10%, 200 mg) in one portion at 40 °C and the mixture was further stirred for 2 h at this temperature. The mixture was filtered on Celite, evaporated to dryness and the residue was dissolved in $CHCl_3$ (40 mL) and washed with brine (20 mL). The organic phase was separated, dried ($MgSO_4$), then PbO_2 (239 mg, 1.0 mmol) was added and O_2 was bubbled through the mixture for 15 min. The mixture was filtered, evaporated, and the residue was purified by flash column chromatography (hexane/EtOAc) to give the title compound **4** as a yellow solid; yield: 2.55 g (58%); mp 48–50 °C; R_f 0.18 (hexane/EtOAc, 2:1).

2,5,5-Trimethyl-2-(3-hydroxyphenyl)pyrrolidin-1-yloxyl Radical (5)

To a stirred solution of compound **4** (876 mg, 4.0 mmol) in 2 M aq HCl (15 mL) was added dropwise a solution of $NaNO_2$ (276 mg, 4.0 mmol) in H_2O (5 mL) at 0 °C. The mixture was stirred at this temperature for 10 min, H_2O (25 mL) was then added and the mixture was warmed up to 40 °C. Then the mixture was stirred for further 30 min at r.t., the pH was adjusted to 7 with $NaHCO_3$ and extracted with $CHCl_3$ (2 × 30 mL). The organic extracts were dried ($MgSO_4$), then PbO_2 (120 mg, 0.5 mmol) was added and O_2 was bubbled through the mixture for 15 min. The mixture was filtered, evaporated, and the residue was purified by flash column chromatography (hexane/EtOAc) to give the title compound **5** as a yellow solid; yield: 240 mg (27%); mp 118–120 °C; R_f 0.32 (hexane/EtOAc, 2:1).

2-[3-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)phenyl]benzo-[*d*]isoselenazol-3-one Radical (6)

To a stirred solution of **4** (1.095 g, 5.0 mmol) in CH_2Cl_2 (20 mL) and Et_3N (1.11 g, 11.0 mmol) was added dropwise a solution of freshly prepared 2-(chloroseleno)benzoyl chloride (1.26 g, 5.0 mmol) in

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Table Compounds **2b**–**25d P**repared

Product[a]	Yield (%)	mp (°C)	IR (nujor or film) ν (cm^{-1})	MS *m/z* (%)
2b	61	oil	1640, 1500 (C=C)	218 (M^+, 34), 204 (11), 145 (14), 132 (100)
2c	89	74−75	1600, 1505 (C=C)	310 (M^+, 5), 296 (2), 80 (7), 91 (100)
2d	44	90−91	1590 (C=C)	310 (M^+, 2), 296 (1), 280 (5), 91 (100)
2f	47	oil	1610, 1500 (C=C)	276 (M^+, 24), 246 (25), 189 (60), 73 (100)
4	58	48−50	3450, 3320 (NH) 1620, 1595 (C=C)	219 (M^+, 11), 205 (6), 189 (6), 133 (100)
5	27	118−120	3150 (OH), 1595 (C=C)	220 (M^+, 13), 206 (10), 190 (9), 134 (100)
6	55	98−100	1640 (C=O), 1575 (C=C)	TSP: 402 $(M + H)^+$
7	73	75−78	1640 (C=C)	302 (M^+, 1), 288 (1), 204 (10), 118 (100)
8	72	oil	3260 (NH) 1740 (C=O), 1590 (C=C)	355 (M^+, 1), 325 (4), 236 (14), 43 (100)
9a	65	97−99	1640 (C=C)	316 (M^+, 8), 302 (7), 115 (30), 41 (100)
9b	73	135−137	3290 (NH) 1640, 1550 (C=C)	297 (M^+, 16), 267 (42), 132 (85), 43 (100)
10	35	198−200	1720 (C=O), 1590 (C=C)	TSP: 328 $(M + H)^+$
11	45	250<	1720 (C=O), 1610 (C=C)	309 (M^+, 11), 295 (14), 279 (60), 41 (100)
12	12	105−107	3230 (OH) 1610, 1585, 1510 (C=C)	220 (M^+, 10), 206 (8), 190 (6), 134 (100)
13	32	149−151	3250 (OH) 1590 (C=C)	220 (M^+, 18), 206 (100), 190 (36), 134 (85)
14	24	90−92	1600 (C=C) 1520 (C=C)	265 (M^+, 19), 235 (11), 179 (29), 162 (100)
15	23	161−163	1600 (C=C) 1520 (C=C)	265 (M^+, 23), 251 (27), 235 (34), 179 (100)
16	17	28−30	1650 (C=O), 1590 (C=C)	234 (M^+,10), 216 (100), 204 (10), 161 (49)
18	60	105−106	3330 (NH) 1620 (C=C), 1550 (NO_2)	354 (M^+, 6), 324 (24), 268 (17), 91 (100)
20	27	190−192	1590 (C=C)	TSP: 409 $(M + H)^+$
21	31	75−77	1620 (C=C)	298 (M^+, 1), 258 (13), 228(24), 172 (100)
23	39	198−201	3400, 3350 (NH) 1670 (C=O), 1575 (C=C)	262 (M^+, 18), 248 (28), 232 (55), 159 (100)
24	47	159−160	1670 (C=O), 1610 (C=C)	TSP: 274 $(M+H)^+$
25a	70	oil	1695 (C=O) 1600 (C=C)	232 (M^+, 15), 218 (25), 202 (11), 146 (100)
25b	82	oil	3410 (OH) 1610, 1500 (C=C)	234 (M^+, 14), 220 (19), 48 (80), 41 (100)
25c	48	38−40	1640, 1600, 1490 (C=C)	298/296 (M^+, 9/9), 284/282 (3/3), 217 (10), 131 (100)
25d	30	108−110	1640, 1600, 1490 (C=C)	328 (M^+, 18), 314 (7), 298 (13), 162 (100)

[a] Satisfactory microanalyses obtained: C ±0.28, H ± 0.27, N ±0.19.

CH_2Cl_2 (20 mL) during 3 min at r.t. and the mixture was stirred for further 45 min. The organic phase was washed with brine (10 mL), dried ($MgSO_4$), filtered, evaporated and the residue was purified by flash column chromatography (hexane/EtOAc) to give the title compound; yield: 1.10 g (55%); mp 98−100 °C; R_f 0.15 (hexane/EtOAc, 2:1).

Chlorosulfonation of Nitroxide Radicals 2a and 2b; General Procedure

To stirred chlorosulfonic acid (10 mL, 0.15 mol) was added dropwise compound **2a** (2.05 g, 10.0 mmol) or **2b** (2.18 g, 10.0 mmol) at 0 °C and the mixture was further stirred for 2 h at r.t. The mixture was poured in portions into crushed ice (100 g) in a 500 mL beaker with stirring and the pH was adjusted to 4 with solid $NaHCO_3$. The mixture was extracted with $CHCl_3$ (2 × 50 mL), the organic phase dried ($MgSO_4$), filtered, evaporated and the residue was further pu-

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rified by flash column chromatography (hexane/Et_2O) to give the title compounds **7a** and **7b** as a mixture of *meta/para* substituted isomers in a 2:1 ratio and **9a**.

2,5,5-Trimethyl-2-(3-chlorosulfonylphenyl)pyrrolidin-1-yloxyl Radical (7a) and 2,5,5-Trimethyl-2-(4-chlorosulfonylphenyl)-pyrrolidin-1-yloxyl Radical (7b)

Yield: 2.22 g (73%); yellow solid; mp 75−78 °C; R_f 0.20 (hexane/Et_2O, 2:1).

2,5,5-Trimethyl-2-(3-chlorosulfonyl-4-methylphenyl)pyrrolidin-1-yloxyl Radical (9a)

Yield: 2.05 g (65%); yellow solid; mp 97−99 °C; R_f 0.23 (hexane/Et_2O, 2:1).

[3-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzenesulfonamidyl]acetic Acid Methyl Ester Radical (8a) and [4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzenesulfonamidyl]acetic Acid Methyl Ester Radical (8b)

To a stirred solution of glycine methyl ester hydrochloride (251 mg, 2.0 mmol) and $NaHCO_3$ (252 mg, 4.0 mmol) in H_2O (10 mL) was added a mixture of compounds **7a** and **7b** (605 mg, 2.0 mmol) in dioxane (5 mL) and stirred for 2 h at r.t. The mixture was extracted with $CHCl_3$ (2 × 10 mL), the organic phase separated, dried ($MgSO_4$), filtered, evaporated and purified by flash column chromatography (hexane/Et_2O, hexane/EtOAc) to give a mixture of **8a** and **8b** as an orange oil; yield: 512 mg (72%); R_f 0.41 ($CHCl_3$/Et_2O, 2:1).

8a

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26, 1.27 (2 s, 2 × 3 H), 1.57 (d, 3 H), 1.66, 1.76, 1.91, 2.03 (4 m, 4 × 1 H), 3.56 (s, 3 H), 3.78, 3.82 (AB m, 2 H, J = 18.0 Hz), 7.42 (dd, 1 H, $J_{H4\text{-}H5} = J_{H6\text{-}H5}$ = 7.7 Hz), 7.69 (m, 2 H), 8.15 (dd, 1 H, $J_{H2\text{-}H4} = J_{H2\text{-}H6}$ = 1.7 Hz).

8b

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.24, 1.28 (2 s, 2 × 3 H), 1.56 (d, 3 H), 1.63, 1.79, 1.88, 2.06 (4 m, 4 × 1 H), 3.61 (s, 3 H), 3.77 (s, 2 H), 7.67, 7.77 (AB m, 4 H, J = 8.5 Hz).

2,5,5-Trimethyl-2-(3-chlorosulfonamido-4-methylphenyl)pyrrolidin-1-yloxyl Radical (9b)

A suspension of **9a** (1.58 g, 5.0 mmol) in 27% aq NH_4OH solution (15 mL) was heated to reflux and allowed to stand overnight at r.t. The solid sulfonamide **9b** (780 mg, 65%) formed was filtered. The aqueous phase was extracted with $CHCl_3$ (10 mL), dried ($MgSO_4$), filtered and evaporated to give a second crop 120 mg (8%) of product as an off-white solid; mp 135−137 °C; R_f 0.27 ($CHCl_3$/Et_2O, 2:1).

4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)-2-sulfonamidobenzoic acid Radical (10)

To a stirred solution of **9b** (594 mg, 2.0 mmol) in aq 2% NaOH solution (10 mL) was added powdered $KMnO_4$ (553 mg, 3.50 mmol) in several portions at 35 °C, then the mixture was allowed to stay at r.t. for 24 h. Oxalic acid was added to decompose the excess $KMnO_4$, the mixture was filtered on Celite, washed with hot water (20 mL) and the filtrate was acidified to pH 2 with 5% aq H_2SO_4. The mixture was extracted with $CHCl_3$ (3 × 20 mL), the organic phase separated, dried ($MgSO_4$), filtered, evaporated and purification by chromatography ($CHCl_3$/Et_2O, $CHCl_3$/MeOH) to afford the title compound **10** as a yellow solid; yield: 230 mg (35%); mp 198−200 °C; R_f 0.21 ($CHCl_3$/MeOH, 5:1).

6-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)-1,1-dioxo-1,2-benzo-[*d*]izothiazol-3(2*H*)-one Radical (11)

Compound **10** (100 mg, 0.3 mmol) was heated cautiously just to the point of melting, then the mixture was allowed to cool to r.t. The residue was further purified by flash column chromatography ($CHCl_3$/MeOH) to give the title compound **11** (42 mg, 45%) as a brown solid; mp > 250 °C; R_f 0.17 ($CHCl_3$/MeOH, 5:1).

Hydrogenolysis of 2c, 2d and 18; General Procedure

To a stirred solution of compound **2c** or **2d** or **18** (20.0 mmol) and HCO_2NH_4 (18.91 g, 0.3 mol) in MeOH (80 mL) was added in one portion Pd/C (10%) (500 mg) at 40 °C. The mixture was stirred and refluxed for 3 h and cooled. H_2O (30 mL) was added and the mixture was filtered on Celite, the filter cake was washed with hot MeOH (2 × 40 mL) and the combined filtrates were evaporated to dryness in vacuo. The residue was dissolved in a mixture of $CHCl_3$ (60 mL) and MeOH (15 mL) and washed with brine (20 mL). The organic phase was dried ($MgSO_4$), PbO_2 (239 mg, 1.0 mmol) was added and O_2 was bubbled through the mixture for 15 min. The mixture was filtered, evaporated to dryness and the residue was purified by flash column chromatography (hexane/Et_2O or $CHCl_3$/Et_2O) to give compound **12** or **13** or **19**.

2,5,5-Trimethyl-2-(4-hydroxyphenyl)pyrrolidin-1-yloxyl Radical (12)

Yellow solid 550 mg (12%); mp 105−107 °C; R_f 0.31 (hexane/EtOAc, 2:1).

2,5,5-Trimethyl-2-(2-hydroxyphenyl)pyrrolidin-1-yloxyl Radical (13)

Yellow solid 1.45 g (32%); mp 149−151 °C; R_f 0.56 (hexane/EtOAc, 2:1).

2,5,5-Trimethyl-2-(3,4-diaminophenyl)pyrrolidin-1-yloxyl Radical (19)

Light sensitive brown solid 1.17 g (25%); mp 126−127 °C; R_f 0.66 ($CHCl_3$/MeOH, 5:1);

All spectroscopic data of compounds **12**, **13** and **19** are the same as described previously.[9]

2,5,5-Trimethyl-2-(2-hydroxy-3-nitrophenyl)pyrrolidin-1-yloxyl Radical (14) and 2,5,5-Trimethyl-2-(2-hydroxy-5-nitrophenyl)pyrrolidin-1-yloxyl Radical (15)

To a stirred solution of compound **13** (1.10 g, 5.0 mmol) in CH_2Cl_2 (25 mL) was added SiO_2/HNO_3 (17% by weight, 2.0 g) in several portions at 10 °C during 45 min while the reaction was monitored by TLC and allowed to warm to r.t. The solution was washed with aq $NaHCO_3$ solution (30 mL), separated, dried ($MgSO_4$), filtered and evaporated to dryness. The residue was purified by flash column chromatography (hexane/Et_2O) to give compound **14** as first band as a red solid (320 mg, 24%); mp 90−92 °C; R_f 0.43 (hexane/Et_2O, 2:1) and compound **15** (315 mg, (23%) as a brownish-green solid; mp 161−163 °C; R_f 0.33 (hexane/EtOAc, 2:1).

2,5,5-Trimethyl-2-(1,4-benzoquinon-2-yl)pyrrolidin-1-yloxyl Radical (16)

To a stirred solution of compound **14** (530 mg, 2.0 mmol) and HCO_2NH_4 (756 mg, 12 mmol) in MeOH (30 mL) was added Pd/C (10%, 100 mg) in one portion at 40 °C and the mixture was further stirred for 2 h at this temperature. The mixture was filtered on Celite and evaporated to dryness. The residue was dissolved in $CHCl_3$ (30 mL), washed with brine (10 mL) and the organic phase was separated and dried ($MgSO_4$). Then PbO_2 (239 mg, 1.0 mmol) was added and O_2 was bubbled through the mixture for 15 min. The mixture was filtered, evaporated, the residue dissolved in freshly prepared 0.1 M aq $AgNO_3$ solution (30 mL) and stirred for 2 h at 40 °C. The pH of the mixture was adjusted to 7 with $NaHCO_3$, filtered on Celite and the filter cake was washed with $CHCl_3$ (2 × 30 mL). The aqueous phase was extracted with $CHCl_3$ (2 × 20 mL), the combined organic phases were dried ($MgSO_4$), then activated MnO_2 (869 mg, 10.0 mmol) was added and O_2 was bubbled through the mixture for 15 min. The mixture was filtered, evaporated and the residue was purified by flash column chromatography (hexane/Et_2O) to give

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compound **16** (80 mg, 17%) as a thick brown oil which solidified on standing; mp 28−30 °C; R_f 0.24 (hexane/Et_2O, 2:1).

2,5,5-Trimethyl-2-(4-benzylamino-3-nitrophenyl)pyrrolidin-1-yloxyl Radical (18)

A solution of compound **17** (2.67 g, 10.0 mmol) and benzylamine (2.14 g, 20.0 mmol) in THF (40 mL) was refluxed for 1 h. After cooling, the solvent was evaporated and the residue dissolved in EtOAc (40 mL). The organic phase was washed with brine (15 mL), dried ($MgSO_4$), filtered, evaporated and the residue was purified by flash column chromatography (hexane/EtOAc) to give compound **18** as an orange solid; yield: 2.15 g (60%); mp 105−106 °C; R_f 0.47 (hexane/EtOAc, 2:1).

2-(2,3-Diphenylquinoxalin-6-yl)-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (20)

A solution of compound **19** (234 mg, 1.0 mmol) and benzil (210 mg, 1.0 mmol) in EtOH (15 mL) was refluxed for 1 h. The solvent was evaporated in vacuo and the residue was purified by flash column chromatography (hexane/Et_2O) to give the title compound **20** as an orange solid; yield: 111 mg (27%); mp 190−192 °C; R_f 0.40 (hexane/EtOAc, 2:1).

2-(2,4-Dimethyl-3*H*-benzo[*b*][1,4]diazepin-7-yl)-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (21)

To a solution of compound **19** (702 mg, 3.0 mmol) in a mixture of 2.0 M aq AcOH (8 mL) and 0.2 M aq NaOAc solution (12 mL) was added pentane-2,4-dione (300 mg, 3.0 mmol) and the mixture was allowed to stand at r.t. for 48 h. The mother liquour was decanted from the solid precipitate and the precipitate was dissolved in $CHCl_3$ (15 mL). The mother liqour was basified with 2.0 M aq NaOH solution (10 mL), extracted with $CHCl_3$ (2 × 10 mL) and the combined organic phases were dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$/MeOH) to give compound **21**; yield: 277 mg (31%); mp 75−77 °C; R_f 0.40 ($CHCl_3$/MeOH, 9:1).

2,5,5-Trimethyl-2-(4-cyano-3-nitrophenyl)pyrrolidin-1-yloxyl Radical (22)

A solution of compound **17** (2.67 g, 10.0 mmol) and Et_4NCN (1.56 g, 10.0 mol) in anhyd MeCN (25 mL) was stirred at 50 °C for 2 h. The solvent was evaporated, the residue dissolved in Et_2O (40 mL) and washed with 5% aq $FeSO_4$ solution (2 × 20 mL) and brine (20 mL). The organic phase was separated, dried ($MgSO_4$), filtered, evaporated and after purification by flash column chromatography (hexane/EtOAc) compound **22** was obtained as a dark red solid; yield: 1.15 g (41%); mp 94−95 °C; R_f 0.14 (hexane/EtOAc, 2:1). All spectroscopic data are the same as described previously.[10]

2-Amino-4-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzamide Radical (23)

To a solution of compound **17** (1.068 g, 4.0 mmol) in MeOH (30 mL) and 30% aq H_2O_2 (5 mL) was added K_2CO_3 (69 mg, 0.5 mmol) and the mixture was allowed to stand at r.t. for 24 h. Then MeOH was evaporated, the residue was extracted with $CHCl_3$ (2 × 20 mL), the organic phase washed with brine (10 mL), dried ($MgSO_4$), filtered and evaporated. The red oily residue was dissolved in MeOH (30 mL), HCO_2NH_4 (1.51 g, 24.0 mmol) was added and the mixture was stirred and heated to 40 °C. Then Pd/C (10%, 100 mg) was added in one portion and the mixture was stirred further for 2 h at this temperature. The mixture was filtered on Celite, evaporated to dryness, the residue dissolved in $CHCl_3$ (40 mL), washed with brine (20 mL) and the organic phase was separated and dried ($MgSO_4$). PbO_2 (239 mg, 1.0 mmol) was then added and O_2 was bubbled through it for 15 min. The mixture was filtered, evaporated, and the residue was purified by flash column chromatography ($CHCl_3$/Et_2O) to give the title compound **23** (420 mg, 39%) as a yellow solid; mp 198−201 °C; R_f 0.78 ($CHCl_3$/Et_2O, 2:1).

7-(1-Oxyl-2,5,5-trimethylpyrrolidin-2yl)-3*H*-benzo[*d*][1,2,3]triazin-4-one Radical (24)

To a stirred solution of compound **23** (262 mg, 1.0 mmol) in 2 M HCl (10 mL) was added dropwise a solution of $NaNO_2$ (69 mg, 1.0 mmol) in H_2O (5 mL) at 0 °C and the mixture was stirred for 10 min at this temperature. It was then allowed to warm to r.t., extracted with $CHCl_3$ (2 × 10 mL) and the combined organic phases were washed with brine, separated, dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography (hexane/EtOAc, $CHCl_3$/Et_2O) to give the title compound **24** as a yellow solid; yield: 130 mg (47%); mp 159−160 °C; R_f 0.42 ($CHCl_3$/Et_2O, 2:1).

4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)-benzaldehyde Radical (25a)

To a stirred solution of compound **2f** (2.76 g, 10.0 mmol) in THF (15 mL) was added aq 2 M HCl (5 mL) and the mixture was stirred for 6 h at r.t. Then Et_2O (20 mL) and brine (10 mL) were added, the organic phase was separated, dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography (hexane/Et_2O) to give compound **25a** as a yellow oil; yield: 1.62 g (70%); R_f 0.41 (hexane/EtOAc, 2:1).

2-(4-Hydroxymethylphenyl)-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (25b)

To a stirred solution of compound **25a** (1.50 g, 6.46 mmol) in EtOH (15 mL) was added $NaBH_4$ (500 mg, 13.21 mmol) and the mixture was stirred for 0.5 h at r.t. The solvent was removed in vacuo and the residue was decomposed with H_2O and extracted with $CHCl_3$ (20 mL). The organic phase was dried ($MgSO_4$), filtered, evaporated and the residue was purified by flash column chromatography (hexane/EtOAc) to give the title compound **25b** as a yellow oil; yield: 1.23 g (82%); R_f 0.48 ($CHCl_3$/Et_2O, 2:1).

2-(4-Bromomethylphenyl)-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (25c)

To a stirred solution of compound **25b** (1.17g, 5.0 mmol) and Et_3N (607 mg, 6.0 mmol) in CH_2Cl_2 (15 mL) was added dropwise $MeSO_2Cl$ (687 mg, 6.0 mmol) dropwise at 0 °C and the mixture was allowed to warm to r.t. The mixture was washed with H_2O (10 mL), the organic phase was separated, dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in acetone (15 mL), LiBr (607 mg, 7.0 mmol) was added and the mixture was stirred and refluxed for 30 min. The solvent was evaporated and the residue was dissolved in Et_2O (20 mL) and the organic phase was washed with H_2O (10 mL). The organic phase was separated, dried ($MgSO_4$), filtered, evaporated and the residue was purified by flash column chromatography (hexane/Et_2O) to give compound **25c** as orange crystals; yield: 0.713 g (48%); mp 38−40 °C; R_f 0.26 (hexane/Et_2O, 2:1).

2-(4-Methanethiosulphonylmethylphenyl)-2,5,5-trimethylpyrrolidin-1-yloxyl Radical (25d)

To a solution of compound **25c** (594 mg, 2.0 mmol) in EtOH (15 mL) and H_2O (5 mL) was added $NaSSO_2Me$ (536 mg, 4.0 mmol) and the mixture was refluxed for 15 min. The solvent was evaporated, the residue dissolved in $CHCl_3$ (20 mL) and washed with brine (10 mL). The organic phase was separated, dried ($MgSO_4$), filtered, evaporated and the residue was purified by flash column chromatography (hexane/EtOAc) to give the title compound **25d** as a pale yellow solid; yield: 196 mg (30%); mp 108−110 °C; R_f 0.21 (hexane/EtOAc, 2:1).

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