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Synthesis of novel DOXYL labelling reagents with electrophilic groups

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Abstract—The oxalic acid salt of 2-aminomethyl-3-oxyl-2,4,4-trimethyl-1,3-oxazolidine has been synthesised from 1-chloroacetone. Being water soluble, the salt is a promising candidate for varied applications. It has been functionalised with electrophilic groups generating novel spin labelling reagents.

Over the last 50 years, nitroxide free radicals have found applications as diverse as redox reagents,[1] SOD mimics,[2] MRI contrast agents[3] and above all as reporter groups[4] (either spin label or spin trap) for probing biological systems, making them a strong and versatile tool accessible to chemists and biophysicists. Despite the vast developments witnessed in the area of spin labels,[5] the syntheses of spin labels possessing the desired blend of properties[6] has always been a challenge to synthetic chemists. In this regard the newer applications of nitroxides, in particular their utility in oxymetry[7] and pH determination[8] of biological systems, has intensified efforts towards the design of novel and more suitable probes possessing properties suitable for these applications.[9]

Spin labelling is rarely achieved by direct construction of a nitroxide group on the biomolecule.[10] The most common methodology for spin labelling involves covalent anchoring of a nitroxide by employing spin labelling reagents,[5a,11] which are functionalised with electrophilic groups or with groups that easily condense with the groups present on biomolecules. The DOXYL (4,4-dimethyl-oxazolidine-*N*-oxyl) spin labels are the prototypical spin labels possessing distinct conformational rigidity, which facilitates the interpretation of rotational correlation times in terms of their local environment. Despite these advantageous properties and the procedural simplicity with which they can be installed at a ketonic site,[10b] DOXYLs have attracted only limited attention from synthetic chemists, probably because the formation of 1,3-oxazolidine rings either suffers from the disadvantage of poor yields for some ketones or is not possible with others.[12] In addition, the oxidation of oxazolidines with *m*-CPBA affords the corresponding DOXYL typically in only about 30% yield (based on the starting oxazolidine).[5a] The literature documents only a few successful preparations of DOXYL based[13] spin labelling reagents. An alternative method of generation of DOXYL from a carboxylic acid, which circumvents the use of a ketone, is also well known.[14,10c,10d] The disadvantage of this method is the relatively greater number of steps required.

With the intention of developing DOXYL based spin labelling reagents, we report herein the synthesis of a novel amino DOXYL spin label, which was conveniently functionalised with electrophilic groups to facilitate the nucleophilic labelling of biomolecules. We sought to prepare an $-NH_2$ group appended to a DOXYL spin label, **1** (Scheme 1), as functionalisation of spin labels with electrophilic groups via reactions with a $-NH_2$ group are well documented.[5a,11a] The disconnection for the amino DOXYL demands that the $-NH_2$ group be blocked, so that the secondary amino group of the 1,3-oxazolidine can be preferentially oxidised over the $-NH_2$ group. The most obvious precursors for the amino blocked 1,3-oxazolidine **2** would be *N*-blocked aminal **3** and α-*N*-blocked amino ketone **4**, as ketones can be conveniently functionalised at an α-position.

Keywords: 4,4-Dimethyl-oxazolidine-*N*-oxyls; Nitroxide; Synthesis; Electrophilic groups; Spin labels.
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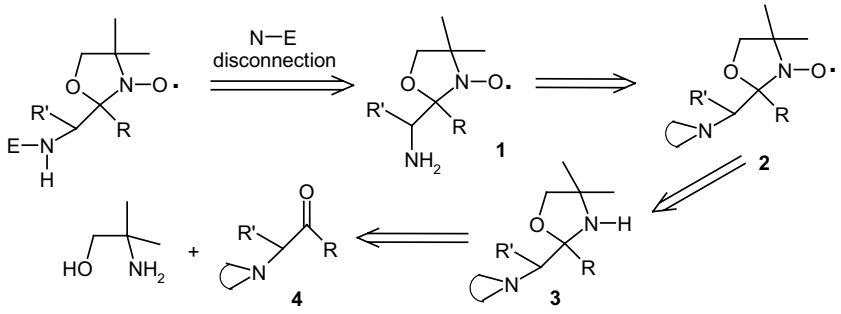
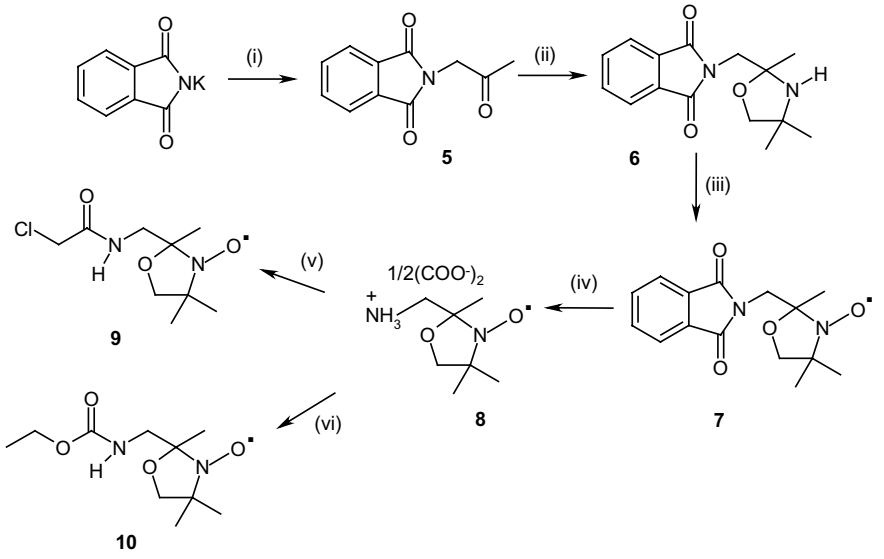


Scheme 1. Strategic disconnection for DOXYL derivatised with electrophilic groups.



Scheme 2. Synthesis of DOXYLs with electrophilic groups. Reagents and conditions: (i) 1-chloroacetone (1.1 equiv), DMF, 0 °C, then rt, 1 h, reflux under nitrogen, 2 h; (ii) 2-amino-2-methyl-1-propanol (3.3 equiv), toluene, $TsOH{\cdot}H_2O$ (cat.), reflux, Dean–Stark water separator, 24 h; (iii) *m*-CPBA (1.2 equiv), CH_2Cl_2, Et_2O, 0 °C, then rt, 3 h; (iv) aq $MeNH_2$ (37%, 5 equiv), Et_2O, rt, 30 min, then aq $(COOH)_2{\cdot}2H_2O$ (0.5 equiv) after removal of $MeNH_2$; (v) Et_3N (1.2 equiv), chloroacetyl chloride (1.1 equiv), 0 °C, then rt, 30 min; (vi) aq 20% NaOH (5 equiv), ClCOOEt (1.1 equiv), 0 °C, then rt, 30 min.

A nucleophilic displacement reaction involving 1-chloroacetone and potassium phthalimide in DMF as per the reported procedure,[15] resulted in the formation of ketone **5**[16] in 92% yield (Scheme 2). The ketone **5** upon condensation with 2-amino-2-methyl-1-propanol with azeotropic removal of water generated oxazolidine **6**[17] in 85% yield. The oxazolidine **6** on oxidation with *m*-CPBA generated phthaloyl DOXYL **7**[18] almost quantitatively (94%). The overall yield of phthaloyl DOXYL **7** from ketone **5** is 73%. To our knowledge, this is the best reported yield for a DOXYL starting from the parent ketone. A biphasic dephthaloylation of **7** in Et_2O with aq $MeNH_2$ followed by subsequent removal of $MeNH_2$ (at 400 mm pressure for 1 h and N_2 bubbling for 3 h) from the aqueous phase and neutralisation of the amino DOXYL with aq oxalic acid generated the oxalate salt of amino DOXYL **8**.[19] With **8** in hand, easy electrophilic functionalisation was illustrated by the formation of *N*-chloroacetyl **9**[20] and *N*-ethoxycarbonyl **10**[21] derivatives in 46% and 55% overall yields, respectively.

The limited solubility of piperidine and pyrrolidine nitroxides has greatly intensified efforts towards the search for newer water soluble nitroxides,[22] which are needed for MRI,[23] protection from oxidative stress and radiative damage[24] and nitroxide mediated controlled free radical polymerisation reactions (NMCPR).[25] Such nitroxides are generally carboxylate[26] or ammonium[27] or sulfonium[22b] salts and are hence highly hydrophilic in nature. We believe that the oxalic acid salt of 2-aminomethyl-3-oxyl-2,4,4-trimethyl-1,3-oxazolidine **8**, will be a valuable addition to the list of known water soluble nitroxide radicals, especially in the light of the search for nitroxides with the charged group separated from the aminoxyl function by a shorter distance.[22b] This is because such nitroxides are expected to be most effective for the NMCPR reactions of hydrophilic monomers in water.

To summarise, we have succeeded in the synthesis of the oxalic acid salt of a novel amino DOXYL spin label, 2-aminomethyl-3-oxyl-2,4,4-trimethyl-1,3-oxazolidine, which could be used for generating potential spin labelling reagents with electrophilic groups, thus allowing nucleophilic labelling of biomolecules with DOXYL spin labels. In addition, it could also be applied to those

reactions/processes in which water-soluble nitroxides are required.

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16. *N*-(2-Oxopropyl)-phthalimide **5**: White crystalline solid, mp 118–120 °C from $CHCl_3$–hexane. Anal. calcd for $C_{11}H_9NO_3$: C, 65.02; H, 4.43; N, 6.90. Found: C, 64.97; H, 4.46; N, 6.86%. IR (KBr): ν_{max} 2969, 1770, 1714, 1417, 1182, 1019, 710 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.27 (s, 3H, C*H*$_3$), 4.50 (s, 2H, C*H*$_2$), 7.71–7.74 (m, 2H, 2×C*H* arom.), 7.84–7.87 (m, 2H, 2×C*H* arom.) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 27.01 (*C*H$_3$), 47.08 (*C*H$_2$), 123.38 (2×*C*H arom.), 131.82 (2×*C* arom.), 134.00 (2×*C*H arom.), 167.39 (2×*C*O), 199.41 (*C*O) ppm.
17. 2-(*N*-Phthalimidomethyl)-2,4,4-trimethyl-1,3-oxazolidine **6**: White amorphous powder, mp 119–121 °C from $CHCl_3$–hexane. Anal. calcd for $C_{15}H_{18}N_2O_3$: C, 65.69; H, 6.57; N, 10.22. Found C, 65.65; H, 6.53; N, 10.17%. IR (KBr): ν_{max} 3357, 2966, 2855, 1708, 1392, 1211, 1062, 726 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ 1.21 (s, 3H, C*H*$_3$), 1.22 (s, 3H, C*H*$_3$), 1.37 (s, 3H, C*H*$_3$), 2.33 (s, 1H, D_2O exchangeable, N*H*), 3.60 (s, 2H, C*H*$_2$), 3.68–3.70 (d, $J = 12$ Hz, 1H, HC*H*), 3.82–3.85 (d, $J = 12$ Hz, 1H, HC*H*), 7.68 (br s, 2H, 2×C*H* arom.), 7.80 (br s, 2H, 2×C*H* arom.) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 26.36 (*C*H$_3$), 27.56 (*C*H$_3$), 28.57 (*C*H$_3$), 45.56 (*C*H$_2$), 59.33 (*C*), 77.24 (*C*H$_2$, overlapped with $CDCl_3$ signal), 96.26 (*C*), 123.38 (2×*C*H arom.), 131.89 (2×*C* arom.), 134.05 (2×*C*H arom.), 168.70 (2×*C*O) ppm.
18. 3-Oxyl-2-(*N*-phthalimidomethyl)-2,4,4-trimethyl-1,3-oxazolidine **7**: Orange yellow powder, mp 85–87 °C from EtOH–H_2O. Anal. calcd for $C_{15}H_{17}N_2O_4$: C, 62.28, H, 5.88; N, 9.69. Found C, 62.32; H, 5.83; N, 9.72%. UV (CH_3CN): λ_{max} 220 nm (ε_{max} 40,240 $dm^3\,mol^{-1}\,cm^{-1}$). IR (KBr): ν_{max} 2974, 2928, 2869, 1722, 1389, 1247, 1067, 724 cm^{-1}. ^1H NMR ($CDCl_3$, Ph–NH–NH_2, D_2O, 300 MHz): δ 0.96 (s, 3H, C*H*$_3$), 1.16 (s, 3H, C*H*$_3$), 1.38 (s, 3H, C*H*$_3$), 3.42–3.45 (d, $J = 8.1$ Hz, 1H, HC*H*), 3.57–3.60 (d, $J = 8.7$ Hz, 1H, HC*H*), 3.74 (s, 2H, C*H*$_2$), 7.55–7.58 (m, 2H, 2×C*H* arom.), 7.72–7.74 (m, 2H, 2×C*H* arom.) ppm. ^{13}C NMR ($CDCl_3$, Ph–NH–NH_2, 300 MHz): δ 19.65 (*C*H$_3$), 20.62 (*C*H$_3$), 26.12 (*C*H$_3$), 44.18 (*C*H$_2$), 61.11 (*C*), 75.22 (*C*H$_2$), 96.89 (*C*), 122.90 (2×*C*H arom.), 131.68 (2×*C* arom.), 133.57 (2×*C*H arom.), 168.38 (2×*C*O) ppm. ESR: 10^{-4} M solution in $CHCl_3$, three equidistant lines with $a_N = 14.5$ G.
19. Oxalic acid salt of 2-aminomethyl-3-oxyl-2,4,4-trimethyl-1,3-oxazolidine **8**: Orange crystals, Anal. calcd for $C_8H_{16}N_2O_4$: C, 47.06; H, 7.84; N, 13.73. Found C, 47.01; H, 7.82; N, 13.69%. UV (CH_3CN): λ_{max} 195 nm (ε_{max} 29,268 $dm^3\,mol^{-1}\,cm^{-1}$). IR (KBr): ν_{max} 3404, 2987, 1617, 1381, 1212 cm^{-1}. ^1H NMR (Ph–NH–NH_2, D_2O, 500 MHz): δ 1.10 (s, 3H, C*H*$_3$), 1.15 (s, 3H, C*H*$_3$), 1.29 (s, 3H, C*H*$_3$), 2.96–3.00 (d, $J = 22$ Hz, 2H, C*H*$_2$), 3.62 (s, 1H, HC*H*), 3.78 (s, 1H, HC*H*) ppm. ^{13}C NMR (D_2O, Ph–NH–NH_2, 500 MHz) δ 19.09 (*C*H$_3$), 20.85 (*C*H$_3$), 25.18 (*C*H$_3$), 44.86 (*C*H$_2$), 61.99 (*C*), 75.78 (*C*H$_2$), 94.97 (*C*), 173.26 (*C*O) ppm. ESR: 10^{-4} M solution in H_2O, three equidistant lines with $a_N = 15.0$ G.
20. 2-(*N*-Chloroacetylaminomethyl)-3-oxyl-2,4,4-trimethyl-1,3-oxazolidine **9**: Orange oil, Anal. calcd for $C_9H_{16}ClN_2O_3$: C, 45.86; H, 6.79; Cl, 15.07; N, 11.89. Found C, 45.82; H, 6.77; Cl, 15.04; N, 11.85%. UV (CH_3CN): λ_{max} 240 nm (ε_{max} 14,461 $dm^3\,mol^{-1}\,cm^{-1}$). IR ($CHCl_3$ film): ν_{max} 3282, 2975, 1675, 1387, 1215 cm^{-1}. ^1H NMR ($CDCl_3$, Ph–NH–NH_2, D_2O, 500 MHz): δ 1.18 (s, 3H, C*H*$_3$), 1.25 (s, 3H, C*H*$_3$), 1.35 (s, 3H, C*H*$_3$), 3.31–3.34 (d, $J = 13.5$ Hz, 1H, HC*H*), 3.44–3.47 (d, $J = 13.5$ Hz,

1H, HC*H*), 3.62–3.64 (d, $J = 7.5$ Hz, 1H, HC*H*), 3.72–3.73 (d, $J = 7.5$ Hz, 1H, HC*H*) and 3.98–4.05 (dd, $J = 15$, 3.5 Hz, 2H, CH_2). ^{13}C NMR ($CDCl_3$, Ph–NH–NH_2, 500 MHz): δ 20.30 (*C*H_3), 21.10 (*C*H_3), 25.71 (*C*H_3), 42.79 (*C*H_2), 46.60 (*C*H_2), 62.32 (*C*), 75.78 (*C*H_2), 97.17 (*C*) and 166.74 (*C*O) ppm. ESR: 10^{-4} M solution in $CHCl_3$, three equidistant lines with $a_N = 14.75$ G.

21. 2-(*N*-Ethoxycarbonylaminomethyl)-3-oxyl-2,4,4-trimethyl-1,3-oxazolidine **10**: Orange yellow oil. Anal. calcd for $C_{10}H_{19}N_2O_4$: C, 51.95; H, 8.23; N, 12.12. Found C, 51.90; H, 8.20; N, 12.11%. UV (CH_3CN): λ_{max} 223 nm (ε_{max} 7104 dm^3 mol^{-1} cm^{-1}). IR ($CHCl_3$ film): ν_{max} 3455, 2997, 1721, 1519, 1370, 1213 cm^{-1}. ^{1}H NMR ($CDCl_3$, Ph–NH–NH_2, D_2O, 300 MHz): δ 1.14 (s, 3H, CH_3), 1.22 (6H, 2×CH_3), 1.31 (s, 3H, CH_3), 3.06–3.10 (d, $J = 14.7$ Hz, 1H, HC*H*), 3.34–3.39 (d, $J = 13.5$ Hz, 1H, HC*H*), 3.56–3.59 (d, $J = 8.4$ Hz, 1H, HC*H*), 3.67–3.70 (d, $J = 8.4$ Hz, 1H, HC*H*), 4.03–4.10 (q, $J = 7.2$ Hz, 2H, CH_2). ^{13}C NMR ($CDCl_3$, Ph–NH–NH_2, 300 MHz): δ 14.57 (*C*H_3), 19.81 (*C*H_3), 20.85 (*C*H_3), 25.50 (*C*H_3), 47.94 (*C*H_2), 60.71 (*C*), 62.05 (*C*H_2), 75.43 (*C*H_2), 97.27 (*C*), 151.05 (*C*O) ppm. ESR: 10^{-4} M solution in $CHCl_3$, three equidistant lines with $a_N = 14.5$ G.

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