Synthesis of New 3,4-Disubstituted 2,5-Dihydro-1*H*-pyrrol-1-yloxyl Spin-Label Reagents via Allylic Rearrangements

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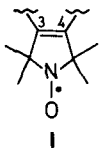
Several 3,4-disubstituted 2,5-dihydro-1*H*-pyrrol-1-yloxyl radicals were synthesized. 2,5-Dihydro-3-hydroxymethyl-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxyl radical (**1**) was reacted with triethyl orthoacetate in a Claisen rearrangement to give the exoolefinic compound **2**. This was converted to its 1-acetoxyl derivative **3**, which was then brominated in the allylic position. Subsequent rearrangement gave the endoolefinic compound 1-acetoxyl-4-bromomethyl-3-ethoxycarbonylmethyl-2,2,5,5-tetramethyl-1*H*-pyrrole radical (**4**). The allylic bromine could be replaced with nucleophiles (NaSCN, KSeCN, thiourea, selenourea, NaN_3 and $KSSO_2Me$) to give **5a–e** and **26**. The diamagnetic thiocyanates and selenocyanates could be reduced with sodium borohydride to the free thiol and selenol monoradicals, which were oxidized to reversible, ester-functionalized disulfide or diselenide diradical reagents **24** and **25**.

The five-membered *N*-oxyl radicals, containing one heteroatom, exhibit significantly higher resistance against various reducing reagents than that of the six-membered less planar piperidin-*N*-oxyl radicals.[1,2] Most of the new spin-label reagents developed in our laboratory bear a reactive functional group(s) attached to a five-membered pyrrolin- or pyrrolidin-*N*-oxyl heterocycle.[3,4] The allylic double bond is of importance both in the reaction steps and in the final product, the spin-label reagent. The reactivity of allylic reagents with or without allylic rearrangement was demonstrated in earlier papers.[5–8] We now report the synthesis of pyrrolin-*N*-oxyls **I** which have reactive substituents at positions-3,4.

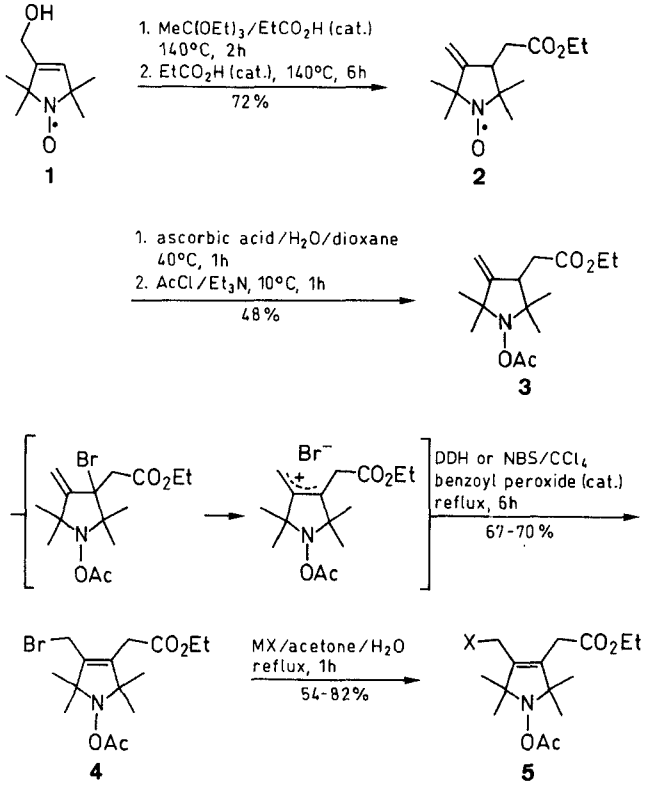


I

To achieve this, the allylic alcohol **1** was reacted in a Claisen rearrangement[9] with ethyl orthoacetate to give ester **2**. Ketone radicals were previously brominated to α-bromo ketones with 2-pyrrolidone hydrotribromide without protection of the *N*-oxyl radical function.[10] However, we found that the allylic bromination of *N*-oxyl radicals is not possible with free radical brominating *N*-bromo amide reagents[11,12] [*N*-bromosuccinimide (NBS) or 1,3-dibromo-5,5-dimethylhydantoin (DDH)], probably because the bromine radical formed undergoes an undesired interaction with the stable *N*-oxyl radical. To overcome this, compound **2** was reduced to the labile *N*-hydroxy ester, which was protected, when acetylated with acetyl chloride, to the diamagnetic *N*-acetoxy derivative **3**.

Compound **3**, containing a diamagnetic terminal double bond, reacts in an allylic rearrangement to give the ω-brominated allylic ester **4**, as the ^1H NMR spectrum

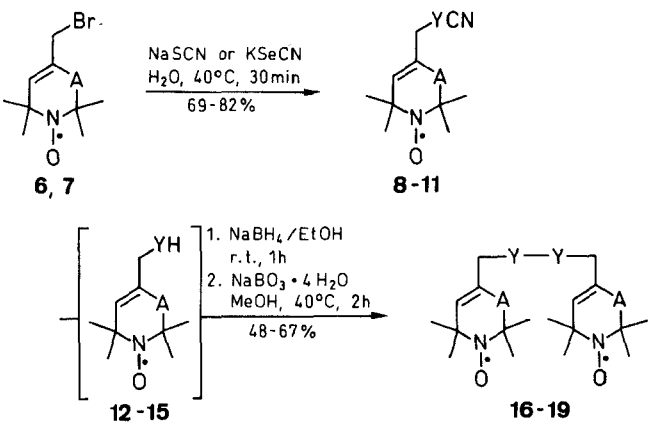
clearly indicates. The high reactivity of the allylic bromine permits the introduction of substituents that react under mild conditions with the functional groups of biomolecules in a nucleophilic replacement reaction. Compound **4** reacted with sodium thiocyanate to give the ω-thiocyanato ester **5a**, with potassium selenocyanate to give the ω-selenocyanato ester **5b**, with thiourea to give the thiuronium salt **5c**, with selenourea to give the selenuronium salt **5d**, and with sodium azide to give the ω-azido ester **5e**.



5	X
a	SCN
b	SeCN
c	$SC(=\overset{+}{N}H_2)NH_2Br^-$
d	$Se(C=\overset{+}{N}H_2)NH_2Br^-$
e	N_3

Scheme 1

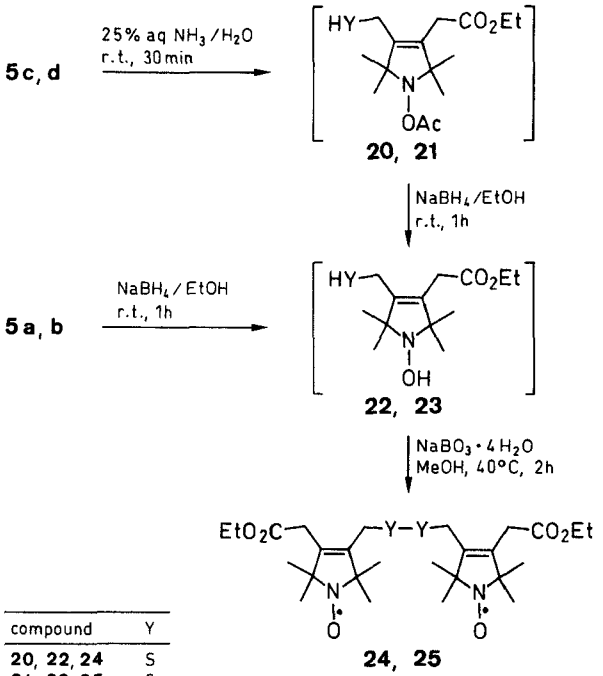
We recently reported the synthesis of 5-membered thiol and selenol monoradicals **12**, **14**, formed by cleavage of thiuronium and selenuronium salts with aqueous ammonia, which were then oxidized to disulfide and diselenide diradicals **16**, **18**,[8] respectively. The monofunctionalized 5- and 6-membered *N*-oxyl thiocyanates **8**, **9** and selenocyanates **10**, **11**, obtained from the corresponding allylic halide **6**, **7**, were reduced with sodium borohydride to thiol and selenol compounds **12**–**15**, which were oxidized to diradical disulfides and diselenides **16**–**19** (Scheme 2). Two of these **16**, **18** have been described[8] and proved to be useful as reversible spin label SH-reagents.[13,14]



	A	Y
6	–	
7	CH_2	
8, 12, 16	–	S
9, 13, 17	CH_2	S
10, 14, 18	–	Se
11, 15, 19	CH_2	Se

Scheme 2

The reaction can be applied to ω-thiocyanato or ω-selenocyanato *N*-acetoxy ester compounds **5a,b**, because the *N*-acetoxy protecting group was also reduced to labile *N*-hydroxy thiol and selenol esters, or alternatively by cleavage of **5c,d** with aqueous ammonia to **20**, **21**, which were reduced with sodium borohydride to **22**, **23**. Solutions of thiol or selenol *N*-hydroxy compounds **22**, **23** are sensitive to oxidation to the diradical when worked up in the presence of air. The oxidation to diradicals **24**, **25** was therefore completed by aeration in the presence of lead dioxide catalyst or stirring with sodium borate in methanol (Scheme 3).



compound	Y
20, 22, 24	S
21, 23, 25	Se

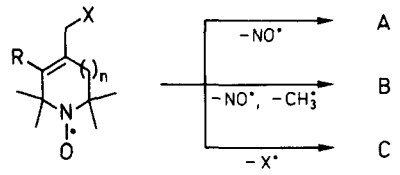
Scheme 3

Table. Compounds **2**–**5e**, **9**–**11**, **17**, **19**, **24**–**28** Prepared

Product	Yield (%)	mp (°C)	Molecular Formula[a]	IR (neat or Nujol) ν (cm^{-1})	MS (70 eV) *m/z* (%)[b]
2	72	41–42	$C_{13}H_{22}NO_3$ (240.3)	1720 (ester C=O), 1650 (C=C)	240 (M^+, 28.6), 210 (A, 61.5), 195 (B, 40.1), 122 (B-CO_2Et, 73), 121 (B-HOCOEt, 100), 107 (122-CH_3, 96.8)
3	48	oil	$C_{15}H_{25}NO_4$ (283.4)	1768 (acetate C=O), 1730 (ester C=O)	–
4	67	oil	$C_{15}H_{24}BrNO_4$ (362.3)	1768 (acetate C=O), 1730 (ester C=O)	–
5a	73	oil	$C_{16}H_{24}N_2O_4S$ (340.4)	2145 (SCN), 1770 (acetate C=O), 1730 (ester C=O)	–
5b	54	oil	$C_{16}H_{24}N_2O_4Se$ (387.3)	2140 (SeCN), 1765 (acetate C=O), 1730 (ester C=O)	–
5c	82	155–157	$C_{16}H_{28}BrN_3O_4S$ (438.4)	3300–2800 (NH_2)	–
5d	76	143–144	$C_{16}H_{28}BrN_3O_4Se$ (485.3)	3300–2800 (NH_2)	–
5e	65	oil	$C_{15}H_{24}N_4O_4$ (324.4)	2100 (N_3), 1760 (acetate C=O), 1720 (ester C=O)	–
9	81	89–90	$C_{11}H_{17}N_2OS$ (225.3)	2140 (SCN)	225 (M^+, 52.6), 180 (B, 15.4), 152 (C–CH_3, 47.5), 121 (B–HSCN, 100), 95 (57.5), 81 (91.3)
10	82	79–80	$C_{10}H_{15}N_2OSe$ (258.2)	2140 (SeCN)	255, 256, 257, 259, 261 (M^+, 2.4, 2.4, 6, 11.8, 2.5), 225–231 (B, Σ11.9), 138 (C–CH_3, 47.5), 123 (A–SeCN, 100), 81 (52)
11	69	119–120	$C_{11}H_{17}N_2OSe$ (238.2)	2140 (SeCN)	269, 270, 271, 273, 275 (M^+, 4.8, 4.5, 12.6, 27.6, 5.1), 152 (C–CH_3, 28.2), 137 (A–SeCN, 44.5), 121 (B–HSeCN, 36.6), 95 (75), 81 (100)
17	52	86–88	$C_{20}H_{34}N_2O_2S_2$ (398.6)	–	398 (M^+, 26.4), 368 (A, 11.8), 152 (C–CH_3, 37.9), 137 (C–CH_3, 100), 95 (37.5), 81 (62.4)
19	67	oil	$C_{20}H_{34}N_2O_2Se_2$ (424.4)	–	410–416 (M^+–Se, Σ50), 152 (C–CH_3, 85), 137 (C–NO, 100), 121 (B–HX, 95.8), 95 (49), 81 (85.3)
24	62	oil	$C_{26}H_{42}N_2O_6S_2$ (542.8)	1730 (ester C=O)	510 (M^+–S, 50.3), 480 (A–S, 21), 224 (C–CH_3, 100), 152 (M^+–X, –CO_2Et, 72)
25	56	oil	$C_{26}H_{42}N_2O_6Se_2$ (636.6)	1732 (ester C=O)	630–642 (M^+, Σ38),[c] 554–562 (M^+–Se, Σ17.3), 239 (C, 30.1), 224 (C–CH_3, 63.2), 152 (C–CO_2Et, 100)
26	54	oil	$C_{16}H_{27}NO_6S_2$ (393.5)	1768 (acetate C=O), 1730 (ester C=O)	393 (M^+, 0.6), 378 (M^+–CH_3, 19), 351 (M–CH_2CO, 4.4), 336 (M^+–CH_3CH_2O, 52.3), 43 (CH_3CO^+, 100)
27	30	oil	$C_{30}H_{48}N_2O_8S_2$ (628.9)	1768 (acetate C=O), 1730 (ester C=O)	613 (M^+–CH_3, 13), 586 (M^+–CH_2CO, 6.6), 555 (M^+–CO_2Et, 4.8), 240 (C–CH_3CO, 53), 226 (C–CH_2CO–CH_3, 100)
28	65	oil	$C_{19}H_{32}N_2O_6S_2$ (448.6)	3370, 3300 (NH_2), 1765 (acetate C=O), 1730 (ester C=O), 1660 (C=C)	–[d]

[a] Satisfactory microanalyses obtained: C ± 0.17, H ± 0.18, N ± 0.15.

[b] Explanation of ion symbols:



M^+ (R = H or CH_2CO_2Et, n = 0, 1)

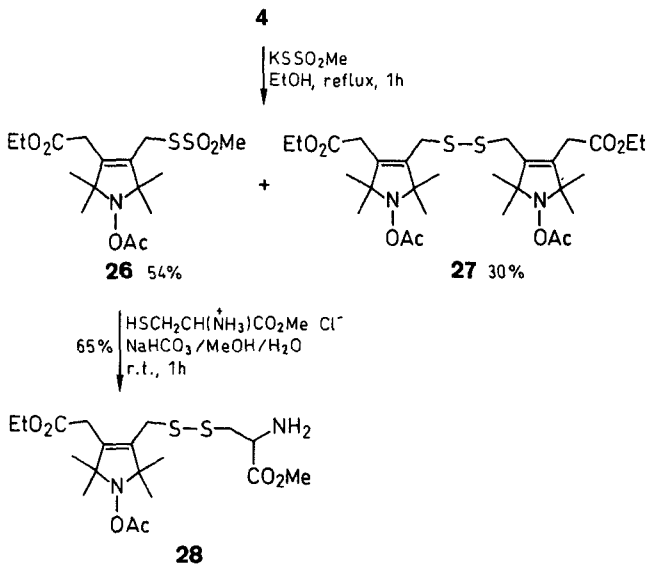
[c] Abundance distribution of the molecular ion cluster agrees with that for $C_{28}H_{46}O_6Se_2$ within ± 10% experimental error.

It was earlier demonstrated[15] that the allylic thiosulfonate, 3-methanesulfonylthiomethyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical, was a reversible thiol label.

The reaction of allylic bromide ester **4** with the potassium salt of methanesulfonothioic acid gave both the thiosulfonate ester **26** and the disulfide **27**. This diamagnetic thiosulfonate ester **26** reacted readily with the SH group of cysteine methyl ester to give the β-disulfido-α-amino acid ester **28** (Scheme 4).

In conclusion, the ester-functionalized allylic bromide **4** is a versatile reagent which may find extensive application in the search for new hetero-bifunctionalized non-reversible and reversible spin label reagents (e.g. when the ester function is converted to a reactive acylating function) for the labelling of biomolecules.

Melting points were determined on a Boetius micro melting point apparatus and are not corrected. Elemental analyses (C, H, N) were performed on a Heraeus Micro U/E apparatus or (Hal) were carried out titrimetrically by Schöniger's method. The IR (Specord 75)



Scheme 4

spectra of the compounds were in each case consistent with the assigned structures. ^1H and ^{13}C NMR spectra were recorded with a Bruker AC-250 spectrometer at 250 and 62.5 MHz, respectively.

The ESR spectra were obtained from 10^{-5} molar solution, using a Zeiss ER 9 spectrometer. All the monoradicals exhibit three equidistant lines and the biradicals show five equidistant lines with $a_N = 14.8-15.0$ G.

The mass spectra were taken on a Finnigan MAT 8430 mass spectrometer/SS300 with data acquisition system. Operation conditions: $U_{acc} = 3$ kV, $E_{el} = 70$ eV, $I_{el} = 0.5$ mA, T_{ion} source = 250 °C, R = 1250. Samples were introduced via the direct insertion probe. The evaporation temperatures of the samples varied between 50 and 250 °C, and were each controlled within ±1 °C accuracy. Assignments were corroborated by high-resolution mass measurements made at R = 10000 by the peak matching technique, with perfluorokerosine as the reference material. FAB: $U_{acc} = 3$ kV, Ion Tech FAB gun at 8 kV, in glycerol/3-nitrobenzyl alcohol (4 : 1) matrix.

Flash column chromatography on silica gel was performed with Merck Kieselgel 60 (0.040–0.063 mm). Qualitative TLC was carried out on commerically prepared plates (20 × 20 × 0.2 cm) coated with Merck Kieselgel GF_{254}.

Compounds **1**[16], **6**[5], **7**[6] and **8**[8] were prepared according to published procedures. The physical and spectral data of all new compounds are listed in the Table.

3-Ethoxycarbonylmethyl-2,2,5,5-tetramethyl-4-methylenepyrrolidin-1-yloxy Radical (2):

A mixture of allylic alcohol **1** (8.51 g, 50.0 mmol), triethyl orthoacetate (81.12 g, 0.5 mol) and propionic acid (0.37 g, 5.0 mmol) was heated at 140 °C with continuous removal of EtOH formed using a Dean-Stark apparatus. After 2 h, further propionic acid (0.37 g, 5.0 mmol) was added and the mixture was heated for 6 h. The excess of orthoacetate was then evaporated off in vacuo. The red-brown residue after purification by flash chromatography (hexane/Et_2O, 2 : 1) gave the ester **2**; yield: 8.64 g (72%); R_f 0.47 (TLC); mp 41–42 °C.

1-Acetoxy-3-ethoxycarbonylmethyl-2,2,5,5-tetramethyl-4-methylenepyrrolidine (3):

To a solution of ester radical **2** (4.81 g, 20.0 mmol) in dioxane (30 mL), a solution of ascorbic acid (10.57 g, 60.0 mmol) in water (10 mL) was added and the mixture was stirred at 40 °C for 1 h. The decolorized solution was evaporated on a rotary evaporator in vacuo, and the residue was then taken up in brine (20 mL) and extracted with $CHCl_3$ (2 × 40 mL). The $CHCl_3$ solution was dried ($MgSO_4$) under Ar. First AcCl (2.36 g, 30.0 mmol) and then slowly Et_3N (3.03 g, 30.0 mmol) were added, the temperature being kept below 10 °C. Stirring was maintained for 1 h, after which the mixture was filtered and the filtrate was evaporated to dryness. The residue was taken up in brine and extracted with Et_2O (3 × 20 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness. The residue was purified by flash chromatography (hexane/Et_2O, 2 : 1), giving the diamagnetic ethyl ester *N*-acetate **3**; yield: 2.72 g (48%); R_f 0.33 (TLC); oil.

^1H NMR ($CDCl_3$/TMS): $\delta = 1.28$ (t, 3H, $J = 6$ Hz, OCH_2CH_3), 0.96, 1.16, 1.25, 1.30 [4 br s, 3H each, $2 \times C(CH_3)_2$], 2.12 (s, 3H, $COCH_3$), 2.42 (d, 1H, $J = 6$ Hz, CH_2CO), 3.02 (t, 1H, $J = 6$ Hz, H-4), 4.17 (q, 2H, $J = 6$ Hz, OCH_2CH_3), 4.82 (br s, 1H, H_{cis}), 4.93 (d, 1H, $J = 2$ Hz, H_{trans}).

1-Acetoxy-4-bromomethyl-3-ethoxycarbonylmethyl-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrole (4):

To a solution of the acetate **3** (2.83 g, 10 mmol) in anhydr. CCl_4 (20 mL) were added the brominating reagent NBS (1.78 g, 10 mmol) or DDH; 1.43 g, 5.0 mmol) and benzoyl peroxide (121 mg, 0.5 mmol) and the mixture was refluxed for 6 h. The reaction was monitored by TLC (hexane/Et_2O, 2 : 1). The succinimide or 5,5-dimethylhydantoin formed was filtered and washed with CCl_4 (10 mL). The filtrate was evaporated to dryness and flash chromatographed (hexane/Et_2O, 2 : 1) to give yield: 2.43 g (67%); R_f 0.27 (TLC); oil.

^1H NMR ($CDCl_3$/TMS): $\delta = 0.97$ (t, 3H, $J = 7$ Hz, OCH_2CH_3), 1.2, 1.4 [2 br s, 2×6H, $2 \times C(CH_3)_2$], 2.18 (s, 3H, $COCH_3$), 3.16 (m, 2H, CH_2O), 4.03 (m, 2H, CH_2Br), 4.15 (q, 2H, $J = 7$ Hz, OCH_2CH_3).

4-Substituted 1-Acetoxy-3-ethoxycarbonylmethyl-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrroles 5a–e; General Procedure:

To a solution of **4** (362 mg, 1 mmol) in acetone (20 mL), the appropriate reagent NaSCN, KSeCN, or NaN_3 or thiourea or selenourea (2 mmol) in water (10 mL) was added and the mixture was refluxed for 1 h, diluted with brine (10 mL), extracted with Et_2O (3 × 20 mL), the combined Et_2O extracts were dried ($MgSO_4$) and evaporated to dryness. The residue was purified by preparative TLC on silica gel (hexane/Et_2O, 2 : 1) to give the title compounds **5a–e**; **5a**: R_f 0.13 (TLC); **5b**: R_f 0.11 (TLC); **5c**: R_f 0.28 (TLC); **5d**: R_f 0.53 (TLC); **5e**: R_f 0.46 (TLC).

5a:

^1H NMR ($CDCl_3$/TMS): $\delta = 1.0$ (t, 3H, $J = 7$ Hz, OCH_2CH_3), 1.2, 1.4 [2 br s, 2×6H, $2 \times C(CH_3)_2$], 2.19 (s, 3H, CH_3), 3.2 (m, 2H, CH_2CO), 3.87 (m, 2H, CH_2SCN), 4.2 (q, 2H, $J = 7$ Hz, OCH_2CH_3).

5b:

^1H NMR ($CDCl_3$/TMS): $\delta = 1.0$ (t, 3H, $J = 8$ Hz, OCH_2CH_3), 1.22, 1.4 [2 br s, 2×6H, $2C(CH_3)_2$], 2.19 (s, 3H, $COCH_3$), 3.17 (br s, 2H, CH_2CO), 3.95 (br s, 2H, CH_2SeCN), 4.2 (q, 2H, $J = 8$ Hz, OCH_2CH_3)

5c:

^1H NMR (DMSO-d_6/TMS): 1.1, 1.2 [2 br s, 2×6H, $2 \times C(CH_3)_2$], 1.2 (t, 3H, $J = 7$ Hz, OCH_2CH_3), 2.2 (s, 3H, $COCH_3$), 3.25 (m, 2H, CH_2CO), 4.0 (br s, 2H, CH_2S), 4.2 (q, 2H, $J = 7$ Hz, OCH_2CH_3).

5d:

^1H NMR (DMSO-d_6/TMS): $\delta = 1.23$ (t, 3H, $J = 7$ Hz, OCH_2CH_3), 1.1, 1.25 [2 br s, 2×6H, $2 \times C(CH_3)_2$], 2.2 (s, 3H, $COCH_3$), 3.25 (m, 2H, CH_2CO), 4.05 (br s, 2H, CH_2Se), 4.2 (q, 2H, $J = 7$ Hz, OCH_2CH_3).

1,2,5,6-Tetrahydro-4-thiocyanatomethyl-2,2,6,6-tetramethylpyridin-1-yloxy Radical (9):

To a solution of **7** (247 mg, 1.0 mmol) in acetone (10 mL) was added NaSCN (162 mg, 2.0 mmol) in H_2O (5 mL). The mixture was stirred at 40 °C for 30 min, then diluted with brine and extracted with Et_2O (2 × 20 mL). The combined Et_2O extracts were dried ($MgSO_4$), filtered and evaporated to dryness. The residue was purified by preparative TLC on silica gel (hexane/Et_2O, 2 : 1), to give the title compound **9**; yield: 182 mg (81%); R_f 0.20 (TLC).

2,5-Dihydro-2,2,5,5-tetramethyl-3-selenocyanatomethyl-1*H*-pyrrol-1-yloxy Radical (10):
The title compound **10** was obtained by the reaction of **6** (233 mg, 1.0 mmol) with KSeCN (216 mg, 1.5 mmol) under the same conditions as for the preparation of **9** above. It was crystallized from $CHCl_3$/hexane; yield: 211 mg (82%); R_f 0.11 (TLC).

1,2,5,6-Tetrahydro-2,2,6,6-tetramethyl-4-selenocyanatomethylpyridin-1-yloxy Radical (11):
The title compound **11** was obtained by the reaction of **7** (247 mg, 1.0 mmol) with KSeCN (216 mg, 1.5 mmol) under the same conditions as for the preparation of **10** above, with subsequent crystallization from $CHCl_3$/hexane; yield: 164 mg (69%); R_f 0.49 (TLC: hexane/EtOAc, 2 : 1).

Disulfide 16, 17 and Diselenide Diradicals 18, 19; Typical Procedure:
To a solution of thiocyanato or selenocyanato compound **8–11** (1.0 mmol) in EtOH (5 mL) was added $NaBH_4$ (76 mg, 2.0 mmol) and the mixture was stirred at r.t. for 1 h. The mixture was diluted with brine (10 mL), extracted with $CHCl_3$ (2 × 10 mL), the combined organic phases were dried ($MgSO_4$) and evaporated to dryness. The residue was taken up in MeOH (10 mL) and $NaBO_3 \cdot 4\,H_2O$ (308 mg, 2 mmol) was added. After stirring at 40 °C for 2 h, the MeOH was evaporated on a rotary evaporator in vacuo. The residue was taken up in $CHCl_3$ (30 mL) and the solution was filtered. The filtrate was evaporated and purified by preparative TLC on silica gel (hexane/Et_2O, 2 : 1) **16**: R_f 0.12 (TLC); **17**: R_f 0.20 (TLC); **18**: R_f 0.18 (TLC); **19**: R_f 0.22 (TLC).

3,3′-Dithio- and 3,3′-Diselenodimethylbis(4-ethoxycarbonylmethyl-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy) Diradicals (24, 25):
From **5a,b**: To a solution of diamagnetic ester **5a,b** (1.0 mmol) in EtOH, was added $NaBH_4$ (113 mg, 3.0 mmol), and the mixture was worked up as above to give the title compounds; **24**: R_f 0.28 (TLC: hexane/EtOAc, 2 : 1); **25**: R_f 0.35 (TLC: hexane/EtOAc, 2 : 1).

From **5c,d**: 25% aq NH_3 (5 mL) was added to an aqueous solution (5 mL) of thiuronium bromide **5c** 438 mg, 1 mmol) or selenuronium bromide **5d** 485 mg, 1 mmol). The mixture was stirred at r.t. for 30 min, saturated with NaCl and extracted with Et_2O (3 × 20 mL). The extract was dried ($MgSO_4$) and evaporated to dryness. The crude diradical **24** or **25** was purified by preparative TLC on silica gel plate with hexane/EtOAc (2 : 1) as eluent.

1-Acetoxy-4-ethoxycarbonyl-2,5-dihydro-2,2,5,5-tetramethyl-4-methanethiosulfonylmethyl-1*H*-pyrrole (26) and 3,3′-Dithiodimethylbis(1-acetoxy-4-ethoxycarbonyl-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy) Diradical (27):
To a solution of the allylic bromide ester **4** (1.81 g, 5 mmol) in EtOH (15 mL) was added $KSSO_2Me$ (900 mg, 6 mmol) and the mixture was refluxed for 1 h, then diluted with brine (20 mL) and extracted with Et_2O. The organic layer was dried ($MgSO_4$), evaporated and chromatographed on silica gel with hexane/EtOAc (2 : 1) as eluent to give products **26** and **27**. **26**: R_f 0.24 (TLC); **27**: R_f 0.37 (TLC).

26:
^{1}H NMR ($CDCl_3$/TMS): δ = 1.27 (t, 3 H, J = 7 Hz, $OCH_2C\underline{H}_3$), 1.2, 1.3 [2 br s, 2 × 6 H, 2 × $C(C\underline{H}_3)_2$], 2.16 (s, 3 H, $COCH_3$), 3.15 (m, 2 H, CH_2CO), 3.35 (s, 3 H, SO_2CH_3), 3.97 (m, 2 H, CH_2S), 4.15 (q, 2 H, J = 7 Hz, $OC\underline{H}_2CH_3$).

27:
^{1}H NMR ($CDCl_3$/TMS): δ = 1.2 (t, 3 H, J = 6.5 Hz, $OCH_2C\underline{H}_3$), 1.2, 1.3 [2 br s, 2 × 6 H, 2 × $C(C\underline{H}_3)_2$], 2.15 (s, 3 H, $COCH_3$), 3.2 (br s, 2 H, CH_2CO), 3.6 (br s, 2 H, CH_2S), 4.17 (q, 2 H, J = 6.5 Hz, $OC\underline{H}_2CH_3$).

1-Acetoxy-4-ethoxycarbonyl-2,5-dihydro-2,2,5,5-tetramethyl-3-[methyldithio(2-amino-2-methoxycarbonylethyl)]-1*H*-pyrrole (28):
To a solution of thiosulfonate ester (**26**; 788 mg, 2 mmol) in MeOH/H_2O (1 : 1; 10 mL), cysteine. HCl methyl ester (378 mg, 2.2 mmol) and $NaHCO_3$ (336 mg, 4 mmol) were added. The mixture was stirred at r.t. for 1 h, diluted with brine (10 mL) and extracted with Et_2O. The ethereal phase was dried ($MgSO_4$), evaporated to dryness and chromatographed on a silica gel plate with $CHCl_3$/EtO (1 : 1) as eluent to give the mixed disulfide of the α-amino acid ester (**28**); R_f 0.30 (TLC).
^{13}C NMR ($CDCl_3$/TMS): δ = 14.1 ($OCH_2\underline{C}H_3$), 19.3 [2 × $C(\underline{C}H_3)_2$], 22.0 ($CO\underline{C}H_3$), 31.6 ($S\underline{C}H_2CH$), 33.8 ($\underline{C}H_2CO$), 34.5 (CH_2S), 52.8 ($CHNH_2$), 53.7 ($CO_2\underline{C}H_3$), 61.1 ($O\underline{C}H_2CH_3$), 70.4, 70.6 [2 × $\underline{C}(CH_3)_2$], 136.2, 136.3 (C=C), 170.3, 171.4, 174.2 (C=O).

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