Synthesis of New 2-Mono- and 2,5-Di-functionalized Pyrrolidin-1-oxyl Spin Labels

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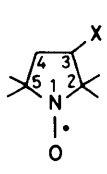
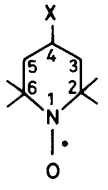
The preparation of new 2-mono- [type (**3**)] and 2,5-di-functionalized [type (**4**)] pyrrolidin-1-oxyl spin labels is described. For the formation of the functional groups (X = OH, CHO, CO_2H, Br), methods previously unused in nitroxide synthesis are applied.

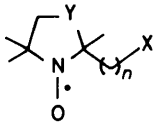
The structures of the nitroxide spin labels most frequently used in biological studies[1,2] have a characteristic feature: the functional groups (X) required for coupling to the biomolecule, are attached at the 3- (**1**) or the 4-position (**2**) of the pyrrolidin- or



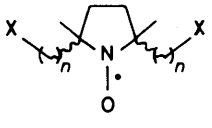
(**1**)



(**2**)



(**3**)



(**4**)

piperidin-1-oxyl ring. We report here the synthesis of new types of nitroxide derivatives (**3**; Y = CH_2) and (**4**) where the short alkyl chains (n = 1—3) containing the reactive groups (X) are attached to the 2- and to the 2,5-carbons respectively. In the case of compound (**3**) the direction of motion of the nitroxide group compared with that of the axis is important in view of the paramagnetism and is different from the molecules (**1**) and (**2**). Thus spin labelling with this new type of nitroxide could be used to give further information about molecular motions. Reactive functional groups were synthesized by methods hitherto unused in nitroxide synthesis, *e.g.* hydroboration with 9-borabicyclo[3.3.1.]nonane (9-BBN), hydroxy halogen change with CBr_4 and Ph_3P, and oxidation with $KMnO_4$–18-crown-6 and with OsO_4–KIO_4. These methods were also employed in the preparation of some difunctional nitroxide derivatives (**4**) which may have use in biological investigations as cross-linking spin labels.

The synthesis of compound (**3**; Y = O, n = 1, X = CO_2H, $CO_2C_6H_4NO_2$-*p*) whose structure is closely related to that of our target molecule (**3**; Y = CH_2) has already been reported.[3] However, the biological application of this type of nitroxide derivative has drawbacks in that because of the oxygen heteroatom it is both more sensitive to reduction and less sensitive to a change in polarity of the medium.

Although difunctional compounds (**4**; n = 2,5,6, X = CO_2H) are also known[4,5] their synthesis involves multi-step formation of the carboxylic group. Our objective was, therefore, to devise a simple, generally applicable method for the preparation of the carboxylic acids where the carboxylic groups are closer to the pyrrolidin-1-oxyl ring [*e.g.* (**3**), (**4**); n = 1, X = CO_2H].

The first step of the synthesis takes advantage of the chemistry developed in the synthesis of proxyl[6] and azethoxyl nitroxides.[7] According to this method, Grignard reagents prepared from allyl and vinyl bromides were allowed to react with 2,2,5-trimethyl-2,3-dihydropyrrole oxide (**5**)[8] followed by Cu^{2+} catalysed aerial oxidation to give compounds (**7a**) and (**7b**) (Method A). Starting with 2,5-dimethyl-2,3-dihydropyrrole oxide (**6**)[9] two successive Grignard addition–aerial oxidation sequences yielded nitroxides with 2,5-diallylpyrrolidine (**7c**) (Scheme 1).

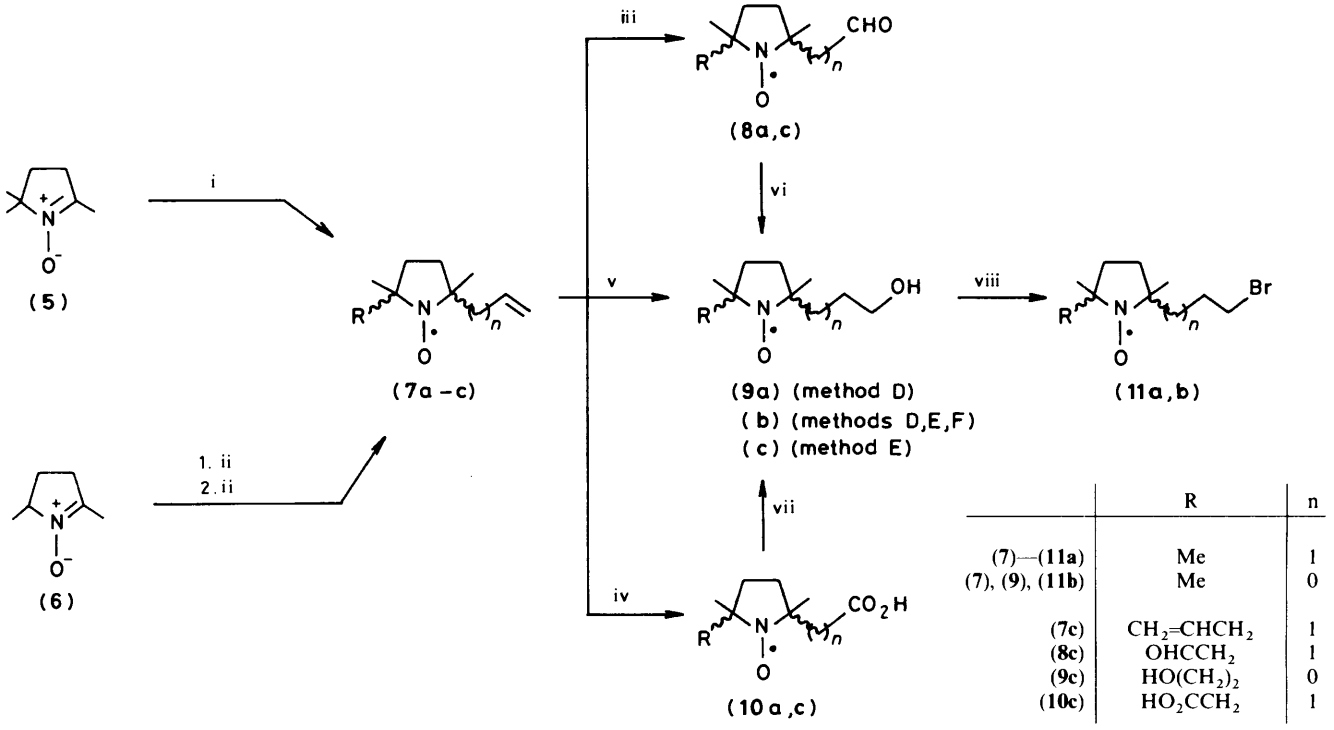
The olefinic groups of compounds (**7a**) and (**7c**) were transformed to aldehyde groups (**8a**) and (**8c**) by osmium tetraoxide-catalysed periodate oxidation[10] (Method B) and to carboxyl groups (**10a**) and (**10c**) by crown ether-catalysed potassium permanganate oxidation in a phase-transfer reaction[11] (Method C). We have recently described the synthesis of compounds (**7c**) and (**10c**) and have also utilized the potassium permanganate oxidation in preparation of various nitroxide fatty acids.[12,13]

The nitroxide alcohols (**9a**—**c**) were synthesized by different methods. Hydroboration of the double bonds of (**7a**) and (**7b**) using 9-BBN as reagent[14] gave the hydroxy derivatives (**9a**) and (**9b**) (Method D). Compounds (**9b**) and (**9c**) were also obtained by the reduction of (**8a**) and (**8c**) with sodium borohydride (Method E). Compound (**9b**) was prepared by the reduction of (**10a**) with borane—dimethyl sulphide[15] (Method F). The substitution of the hydroxy groups of (**9a**) and (**9b**) for bromide (**11a**) and (**11b**) was carried out by means of carbon tetrabromide and triphenylphosphine reagents[16] (Method G).

It was possible to separate the *cis*/*trans* isomers of compounds (**7c**) and (**9c**), using column chromatography. In accordance with an earlier and analogous reaction[7] we supposed that the allylic Grignard reagents preferred attack on the nitrone (**6**) from the less hindered side, hence we tentatively assigned *trans*-geometry for the major isomer and *cis*-geometry for the minor. The separation of the isomers on a preparative scale failed but the ratio of the *trans*/*cis* isomers (2:1) of (**7c**) was determined by h.p.l.c.

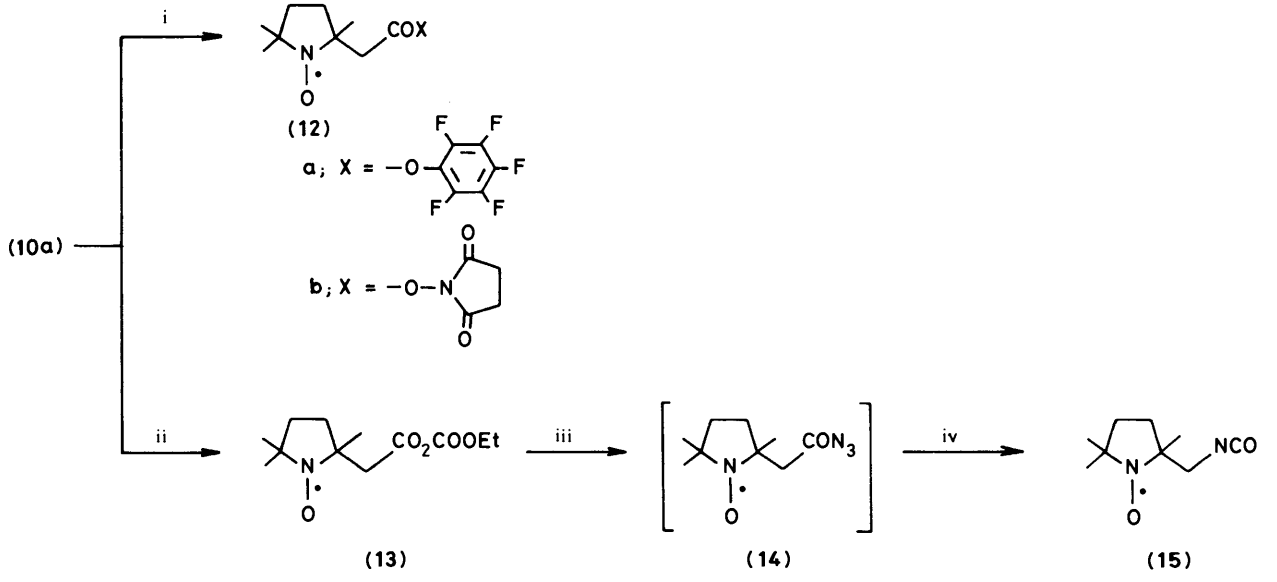
To increase the reactivity of the carboxylic acid (**10a**) as an acylating agent some of its reactive derivatives were also prepared (Scheme 2). Thus the pentafluorophenyl (**12a**) and the *N*-hydroxysuccinimide active esters (**12b**) were obtained using *N*,*N*′-dicyclohexylcarbodi-imide (DCC) as a coupling reagent (Method H).

Compound (**10a**) gave acyl carbonate (**13**) with ethyl chloroformate in the presence of triethylamine (Method I). Following a known procedure[17] first the acyl azide (**14**) was obtained from (**13**) with sodium and without isolation it was converted into the nitroxide isocyanate compound (**15**) in a Curtius reaction (Method J).



	R	n
(7)—(11a)	Me	1
(7), (9), (11b)	Me	0
(7c)	CH_2=$CHCH_2$	1
(8c)	$OHCCH_2$	1
(9c)	$HO(CH_2)_2$	0
(10c)	HO_2CCH_2	1

Scheme 1. *Reagents:* i, CH_2=$CH(CH_2)_nMgBr$, Cu^{2+}, O_2 (Method A); ii, CH_2=CH–CH_2MgBr, Cu^{2+}, O_2 (Method A); iii, KIO_4, OsO_4 (Method B); iv, $KMnO_4$, 18-crown-6 (Method C); v, 9-BBN, 30% H_2O_2 (Method D); vi, $NaBH_4$ (Method E); vii, Me_2S·BH_3 (Method F); viii, Ph_3P, CBr_4 (Method G).



Scheme 2. *Reagents:* i, *N*-Hydroxysuccinimide or pentafluorophenol, DCC (Method H); ii, $ClCO_2Et$, TEA (Method I); iii, NaN_3 (Method J); iv, heat in hexane

Experimental

M.p.s were determined on a Boetius micro melting-point apparatus. I.r. spectra were obtained neat or as Nujol mulls on a Carl-Zeiss Specord 75 spectrometer. E.s.r. spectra were obtained for 10^{-4} molar solutions ($CHCl_3$, MeOH) using a Carl-Zeiss ER-9 spectrometer. All of the monoradicals exhibit three equidistant lines with a_N = 14.3—14.5 G. Mass spectra were recorded with a Varian-MAT-SM-1 instrument. T.l.c. was carried out on Merck silica gel 60 F_{254} plates. H.p.l.c. was carried out on a 15 cm long 4.6 mm i.d. column packed with Chromsil 6 μm packing using dichloromethane–hexane (45:55 v/v) as eluant with a 2.0 ml/min flow rate; detection was at 236 nm. After work-up of the reaction mixtures the organic extracts were dried over sodium or magnesium sulphate. Light petroleum refers to the fraction b.p. 40—70 °C. The two starting nitrone compounds (**6**)[8] and (**7**)[9] were prepared by known methods. The new compounds are presented in the Table.

Table. Preparation and physical data of nitroxides (7)—(15)

Compd.	Yield (%)	Method	M.p. (°C) or b.p. (°C)/mmHg	R_F	Molecular formula	Found (%) (Required) C	H	N	$\nu_{max.}$ (cm^{-1})	*m/z* (Rel. int.)
(7a)	83	A	93—94/13	0.38[a]	$C_{10}H_{18}NO$	71.30 (71.39)	11.00 (10.78)	8.10 (8.32)	1 635 (C=C)	—
(7b)	81	A	80—83/14	0.3[a]	$C_9H_{16}NO$	70.40 (70.09)	10.20 (10.46)	9.35 (9.08)	1 630 (C=C)	(M^+ + 1) 155 (5.6), (M^+) 154 (25), 140 (15.2), 127 (70), 112 (32), 68 (100)
(7c) *trans*	36[b]	A	Oil	0.28[a]	$C_{12}H_{20}NO$	74.00 (74.18)	10.15 (10.38)	7.15 (7.31)	1 640 (C=C)	(M^+ + 1) 195 (13.5), (M^+) 194 (100), 153 (46), 67 (54)
(7c) *cis*		A		0.23[a]	$C_{12}H_{20}NO$	74.00 (74.18)	10.30 (10.38)	7.70 (7.31)	1 640 (C=C)	(M^+ + 1) 195 (15), (M^+) 194 (98), 153 (44), 67 (100)
(8a)	39	B	Oil	0.37[c]	$C_9H_{16}NO_2$	64.05 (63.50)	9.30 (9.47)	8.25 (8.23)	1 715 (CO)	—
(8c)	59	B	Oil	0.31[d]	$C_{10}H_{16}NO_3$	60.35 (60.59)	8.05 (8.14)	7.00 (7.07)	1 720 (CO)	(M^+ + 1) 199 (10), (M^+) 198 (19), 184 (8.8), 156 (100), 127 (50.5)
(9a)	47	D	Oil	0.39[e]	$C_{10}H_{20}NO_2$	64.20 (64.48)	10.70 (10.82)	7.55 (7.52)	3 100—3 600 (OH)	—
(9b)	45	D	Oil	0.36[e]	$C_9H_{18}NO_2$	63.00 (62.76)	10.50 (10.53)	8.35 (8.13)	3 000—3 600 (OH)	—
	41	E								
	22	F								
(9c) *trans*	42[b]	E	Oil	0.54[f]	$C_{10}H_{20}NO_3$	59.30 (59.38)	9.85 (9.97)	6.65 (6.92)	3 390 (OH)	(M^+ + 1) 203 (5), (M^+) 202 (34) 158 (100), 113 (29), 68 (40)
(9c) *cis*		E		0.50[f]	$C_{10}H_{20}NO_3$	59.10 (59.38)	10.00 (9.97)	6.70 (6.92)	3 380 (OH)	(M^+ + 1) 203 (7), (M^+) 202 (53.5), 158 (100), 113 (26), 68 (38)
(10a)	72	C	Oil	0.62[g]	$C_9H_{16}NO_3$	58.05 (58.05)	8.80 (8.66)	7.65 (7.52)	1 725 (CO)	(M^+ + 1) 187 (2), (M^+) 186 (14), 154 (21), 127 (21), 127 (26), 57 (100)
(10c)	13	C	Oil	0.24[h]	$C_{10}H_{16}NO_5$	52.20 (52.17)	7.10 (7.01)	6.50 (6.08)	1 715 (CO)	(M^+ + 1) 231 (1.2), (M^+) 230 (12) 212 (33.5), 198 (26), 171 (66), 154 (84), 112 (100)
(11a)	52	G	96—98	0.32[i]	$C_{10}H_{19}BrNO$	48.20 (48.20)	8.00 (7.69)	5.55 (5.62)	—	—
(11b)	55	G	Oil	0.29[i]	$C_9H_{17}BrNO$	45.75 (45.97)	7.40 (7.29)	5.60 (5.96)	—	—
(12a)	61	H	62—65	0.33[a]	$C_{15}H_{15}NO_3F_5$	50.80 (51.14)	4.35 (4.29)	3.75 (3.98)	1 780 (CO)	(M^+ + 1) 353 (0.6). (M^+) 352 (6), 184 (10), 127 (31) 32 (100)
(12b)	63	H	70—72	0.43[j]	$C_{13}H_{19}N_2O_5$	54.95 (55.11)	6.90 (6.76)	9.95 (9.89)	1 805, 1 770 1 730 (CO)	—
(13)	92	I	Oil	0.36[j]	$C_{12}H_{20}NO_5$	55.75 (55.80)	7.60 (7.80)	5.60 (5.42)	1 820, 1 780 (CO)	—
(15)	79	J	Oil	0.58[d]	$C_9H_{15}N_2O_2$	58.95 (59.00)	8.40 (8.25)	15.00 (15.29)	2 250 (NCO)	—

[a] Eluant: hexane–ethyl acetate (10:1). [b] Total yield of isomers. [c] Eluant: hexane–ethyl acetate (2:1). [d] Eluant: hexane–ethyl acetate (1:1). [e] Eluant: hexane–ethyl acetate (1:2). [f] Eluant: chloroform–methanol–acetone (5:1:1). [g] Eluant: ether–light petroleum–acetic acid (4:1:0.1). [h] Eluant: hexane–tetrahydrofuran–acetic acid (4:2:0.1). [i] Eluant: chloroform–methanol (10:1). [j] Eluant: chloroform–methanol (1:1).

General Procedure for the Preparation of Nitroxides Containing Allyl and Vinyl Groups (**7a**—**c**).—*Method A.* The nitrone (**5**) or (**6**) (0.1 mol) was added to a Grignard reagent prepared from magnesium (3.64 g, 0.15 mol) and allyl or vinyl bromide (0.15 mol) in ether (100 ml). The solution was stirred for 2 h at room temperature and then treated with saturated aqueous ammonium chloride (5 ml) and water (5 ml). The ether phase was separated, washed with saturated brine, and evaporated. The residue was taken up and stirred in methanol (200 ml) containing concentrated aqueous ammonium hydroxide (5 ml) and copper(II) acetate (0.5 g). Oxygen was bubbled through the reaction mixture until the solution developed a deep blue colour (indicating the appearance of **7a** or **b**). Following evaporation of the solvent, the residue was taken up in chloroform (50 ml) and the solution washed with saturated aqueous sodium hydrogen carbonate and water, dried and evaporated. The residue was then distilled to give the product. In synthesis of (**7c**) the residue (a new nitrone compound) was not purified but was dried by azeotropic removal of water with benzene, and added to the Grignard reagent prepared from magnesium (3.64 g, 0.15 mol) and allyl bromide (0.15 mol). After being stirred for 2 h the reaction mixture was worked up as above. Silica gel chromatography of the residue with hexane–ethyl acetate (10:1) as the eluant afforded pure (**7c**) as a red oil. It was possible to separate the *cis*/*trans* isomers in quantities sufficient for analysis. Since on a preparative scale the separation was not possible, the following reactions were carried out with the mixture of the isomers.

General Procedure for the Preparation of Nitroxide Aldehydes (**8a**, **c**).—*Method B.* To a solution of (**7a**, **c**) (10 mmol) in dioxane–water (30 ml:10 ml) was added osmium tetraoxide [25 mg for (**8a**) or 50 mg for (**8c**)]. After being stirred for 5 min the reaction mixture turned brown. Potassium periodate [4.6 g, 20 mmol for (**8a**) or 9.2 g, 40 mmol for (**8c**)] was added in small portions during 30 min, and the mixture was stirred for an additional 2 h after which the colour changed back to yellow. The mixture was extracted with ether (3 × 30 ml), and the extracts dried and evaporated. The residue oils were chromatographed on a silica gel column using hexane–ethyl acetate [(2:1) for (**8a**) and (1:2) for (**8c**)] as the eluant. Since it was not possible to separate the *cis*/*trans* isomers of the nitroxide dialdehyde (**8c**) even by t.l.c., the mixture of isomers was used for the next reaction.

General Procedure for the Preparation of Nitroxide Carboxylic Acids (**10a** *and* **c**) *by the Oxidation of the Terminal Double Bond.—Method C.* Compound (**7a**, **c**) (15 mmol) was added to a suspension of potassium permanganate [(7.1 g, 45 mmol for (**10a**) or 14.2 g, 90 mmol for (**10c**)] and 18-crown-6 [0.8 g, 3 mmol for (**10a**) or 1.6 g, 6 mmol for (**10c**)] in benzene. The reaction mixture was stirred for 48 h, and then filtered and washed with 5% aqueous sodium hydroxide and water. The combined water washings were acidified with hydrochloric acid, extracted with chloroform, and the extract dried and evaporated. The residue was chromatographed on a silica gel column with ether–light petroleum–acetic acid (3:1:0.1) as the eluant.

General Procedures for the Preparation of Nitroxide Alcohols (**9a**—**c**).—*Method D: Preparation of* (**9a** *or* **b**) *by hydroboration of* (**7a**,**b**). To the corresponding nitroxide (**7a**,**b**) (10 mmol) was added dropwise an 0.5M tetrahydrofuran solution of 9-BBN (40 ml, 20 mmol). The reaction mixture was stirred for 1 h. Aqueous sodium hydroxide (19%, 8 ml, 20 mmol) and aqueous hydrogen peroxide (30%, 7 ml) were then added carefully at 0 °C. The reaction mixture was saturated with potassium carbonate and extracted with ether. The organic extracts were combined, dried, and evaporated and the residue was chromatographed on silica gel column with hexane–ethyl acetate (1:2) as eluant to give the nitroxide hydroxy compounds (**9a**) and (**9b**).

Method E: Reduction of (**8a**,**c**) *to* (**9b**,**c**) *with sodium borohydride.* To a solution of (**8a**,**c**) (10 mmol) in ethanol (50 ml) was added sodium borohyride [1 g for (**9b**), 2 g for (**9c**)]. The reaction mixture was stirred at room temperature for 1 h after which the ethanol was evaporated and water (15 ml) was added. After extraction with chloroform (3 × 20 ml), the combined organic phases were dried and evaporated and the residue chromatographed on a silica gel column with chloroform–methanol (100:1) as eluant to give the hydroxy compounds (**9b**,**c**). The *cis*/*trans* isomers of nitroxide alcohol (**9c**) were separated in quantities sufficient only for analytical determination.

Method F: Reduction of (**10a**) *to* (**9b**) *with borane–dimethyl sulphide.* To a stirred solution of (**10a**) (3 mmol) in ether (20 ml) was added dropwise the borane–dimethyl sulphide complex (2.0M in THF; 4 mmol). The reaction mixture was stirred at room temperature for an additional 2 h, after which methanol (60 ml) was added, and stirring continued for 4 h. After evaporation of methanol, the residue was chromatographed on a silica gel column with hexane–acetate (1:2) as the eluant.

General Procedure for the Preparation of Nitroxide Halides (**11a**,**b**).—*Method G.* To a stirred solution of the nitroxide alcohol (**9a**,**b**) (5 mmol) in dichloromethane (20 ml) were added triphenylphosphine (2.62 g, 0.01 mol) and carbon tetrabromide (3.36 g, 0.01 mol). The reaction mixture was stirred and refluxed for 2 h and then evaporated. The residue was suspended in light petroleum, the suspension filtered, and the precipitated triphenylphosphine oxide was washed with hot light petroleum. The combined filtrates were evaporated and the residue chromatographed on a silica gel column with hexane–ethyl acetate (10:1) as the eluant.

General Procedures for the Preparation of Active Derivatives of the Carboxylic Acid (**10a**).—*Method H: Active esters* (**12a**,**b**) *of* (**10a**): To a stirred solution of (**10a**) (0.186 g, 1 mmol), in dry ethyl acetate (20 ml) was added at 0 °C the corresponding hydroxy compound [for (**12a**): pentafluorophenol (0.184 g, 1 mmol); for (**12b**): *N*-hydroxysuccinimide (0.115 g, 1 mmol)] and *N*,*N*′-dicyclohexylcarbodi-imide (0.206 g, 1 mmol). The reaction mixture was stirred at 25 °C for 3 h and then filtered. The filtrate was washed with saturated aqueous sodium hydrogen carbonate, dried, and chromatographed on a silica gel column with carbon tetrachloride–ether (50:1) as eluant for (**12a**) and chloroform–ether (50:2) as eluant for (**12b**).

Method I: Acyl Carbonate (**13**) *from* (**10a**). To a stirred solution of (**10a**) (0.93 g, 5 mmol) in ether (20 ml) were added at 0 °C triethylamine (0.5 g, 5 mmol) and ethyl chloroformate (0.54 g, 5 mmol). The reaction mixture was stirred at 20 °C for 3 h and then filtered. The filtrate was washed with saturated aqueous sodium hydrogen carbonate, dried, and evaporated to yield a chromatographically (t.l.c.) pure red oil as the product.

Method J. Nitroxide isocyanate (**15**) *from* (**13**). To a stirred solution of (**13**) (0.51 g, 2 mmol) in acetone (10 ml) was added at 0 °C a solution of sodium azide (0.195 g, 3 mmol) in water (5 ml). The acetone was evaporated off at 0 °C under reduced pressure. The residue was extracted with hexane, and the extracts were dried, refluxed for 30 min, and evaporated to give chromatographically pure (t.l.c.) oily products.

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