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Efficient S-alkylation of cysteine in the presence of 1,1,3,3-tetramethylguanidine

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ABSTRACT

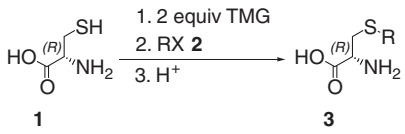
The synthesis of S-alkylated cysteine derivatives was carried out successfully in the presence of 1,1,3,3-tetramethylguanidine. Alkylation proceeded in high yields on unprotected amino acids and peptides containing a sulfhydryl group.

The isoprene unit located at the C-terminal of cysteine has been found to be crucial for membrane association and transforming the activity of oncogenic *ras* proteins.[1–5] The need for this post-translational modification of L-cysteine residue, the necessity for alkylation of sulfhydryl groups in peptide mapping and protein identification,[6] and the wide spectrum of biological activity of molecules with a cysteine moiety in their structure[7–11] have inspired research on the S-alkylation problem in cysteine chemistry.

The reported conditions for S-alkylation of cysteine[12–15] with primary alkyls (dilute solutions: NaOH or KOH in water and alcohol, or EtONa in ethanol) which were useful in substitution reactions with primary alkyl halides, failed in the case of long chain lipophilic halides. Alkylation of the sulfhydryl group in proteins with farnesyl bromide in alcohol/water mixture in the presence of MgO has been reported.[16,17] Unprotected and protected cysteines were alkylated in DMSO/DMF/MeCN using a fourfold excess of farnesyl bromide in the presence of *N*,*N*-diisopropylethylamine (DIEA).[18] Brown et al. prepared alkylated cysteine derivatives in an ammonia/methanol solution or in liquid ammonia;[19] these procedures were applied for the synthesis of molecules in anti-leukemia drug research,[20,21] lipidated peptides,[22] and S-farnesylated-N-acylated-L-cysteine.[23] S-alkylated L-cysteines[24] containing a protected carboxyl or amino group were prepared in the presence of a fresh solution of sodium ethoxide in absolute ethanol. Unsatisfactory yields, poor selectivity, problems with solubility and purification, formation of side-products, racemisation etc. were among the difficulties encountered.

Herein we present a very simple and effective method for the S-alkylation of L-cysteine in the presence of 1,1,3,3-tetramethylguanidine (TMG) which is free from the above mentioned problems (Scheme 1).

TMG is a strong, non-ionic base which is widely applied in various base-mediated organic reactions.[25,26] However, to our knowledge, its application in reactions of amino acids[27–31] and the S-alkylation of thiols[32–34] is very limited.



Scheme 1.

We observed that treatment of L-cysteine in lower alcohols (methanol, ethanol, propanol) with two equivalents of TMG resulted in an exothermic reaction leading to a very reactive and highly soluble di-salt. In fact, preparation of solutions up to a concentration of 40% was possible. Addition of alkylating agents to the homogeneous, concentrated solution resulted in exothermic S-alkylations. In order to complete the reaction, the mixture was heated at 50 °C, then (after TLC or GC analysis[35]) the solvent was evaporated; water and an equivalent of acetic acid or HCl was added to the residue and the resulting S-substituted L-cysteine precipitate was filtered, washed with water, (if insoluble in water) and recrystallized from ethanol or dilute ethanol—Method A. For products soluble in water an equivalent of acetic acid or HCl (in methanol or ethanol) and small amount of methanol or other alcohol to initiate of crystallization were added—Method B. TMG could be recovered from the reaction mixture by concentration, basification to pH 12 with 30% NaOH at a temperature of 5–10 °C, extraction with chloroform, washing with brine, drying over $MgSO_4$, concentration by evaporation, and distillation under reduced pressure to afford the pure base which could be recycled in >83% yield.

Using this procedure[37] the alkylation of L-cysteine with a series of alkyl halides was carried out. The results are given in Table 1. The yields of isolated products were very high, even for *trans*,-*trans*-farnesyl, *trans*-geranyl or dodecyl bromides, and in some cases yields were almost quantitative. A lower yield was observed for highly soluble *S*-*tert*-butyloxy carbonylmethyl-L-cysteine entry m. No O- or N-alkylated side-products were detected in the reaction mixture if strictly stoichiometric quantities of reagents were used and the homogeneity of cysteine di-salt solution prior to alkylation was assured.

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Table 1
Preparation of S-alkylated sulfides 3a–**q** in the presence of TMG and comparison with the best results from the literature

Entry	Thiol **1**	Alkylation halide **2**	Mp [°C]	Yield[a] [%]	Lit. yield[36] [%]	Lit. conditions
a	L-Cysteine	Methyl iodide	219–220	93[f]	88[36a]	EtONa/EtOH
b	L-Cysteine	Ethyl bromide	253–254	89[f]	75[36b]	$NaNH_2/NH_{3(liq)}$
c	L-Cysteine[b]	Benzyl chloride	216–218	91[e]	94[15]	NaOH(aq)/EtOH
d	L-Cysteine	*p*-Methylbenzyl chloride	210–211	95[e]	—[36c]	$Et_3N/EtOH/H_2O$
e	L-Cysteine	*p*-Nitrobenzyl chloride	154–156	99[e]	60[36d]	$NaOH_{(aq)}$/1,4-dioxane
f	L-Cysteine	Propargyl chloride	178–179	85[e]	85[36e]	$Ba(OH)_{2(aq)}$/EtOH
g	L-Cysteine[b]	Butyl iodide	192–194	88[e]	75[36b]	$NaNH_2/NH_{3(liq)}$
h	L-Cysteine	*trans*,*trans*-Farnesyl bromide	170-172	91[f]	91[36f]	$NH_{3(g)}$/MeOH
i	L-Cysteine	*trans*-Geranyl bromide	155-157	92[f]	75[36g]	$NaNH_2/NH_{3(liq)}$
j	L-Cysteine	Dodecyl bromide	214–215	89[e]	80[13]	$NaOH_{(aq)}$/EtOH
k	L-Cysteine[b]	1,4-Dichloro-2-butyne[c]	173–175	79[e]	—	—
l	L-Cysteine	2-Bromoacetophenone	94–96	93[e]	80[36h]	$KOH_{(aq)}/EtOH/H_2O$
m	L-Cysteine	*t*-Butyl chloroacetate	179–180	53[f]	—	—
n	L-Cysteine	Chloroacetonitrile	160–162 dec.	93[f]	—	—
o	D-Penicillamine	2-Bromomethylnaphthalene	176-179	91[f]	—	—
p	D-Penicillamine	Benzyl chloride	163–165	84[e]	79[36i]	$NH_{3(liq)}$
q	L-Glutathione red[d]	*p*-Nitrobenzyl chloride	199–200	99[f]	74[36j]	$NaOH_{(aq)}/EtOH/H_2O$

[a] Yield refers to isolated products—not optimized.
[b] L-Cysteine hydrochloride monohydrate and 3 equiv of TMG were used.
[c] 0·5 equiv of **2k** were used.
[d] 3 equiv of TMG were used.
[e] Isolation method A.
[f] Isolation method B.

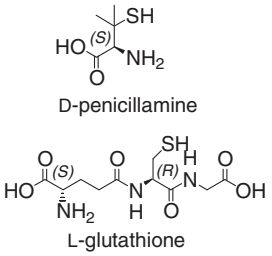

Figure 1.

There was no analytical evidence for racemisation occurring during the alkylation. The same results were obtained when commercially available L-cysteine hydrochloride was used but one additional equivalent of TMG had to be added. Due to the mild reaction conditions a wide range of alkylating agents containing labile functional groups such as esters, ketones or nitriles could be used. Thus TMG is an ideal basic reagent for the discrimination between the three nucleophilic centers of the cysteine di-anion and leads to selective alkylation of the sulfhydryl group. The S-alkylation reaction was extended successfully to D-penicillamine (Fig. 1) (entries o and p) and a more complex compound, the important tripeptide, L-glutathione (entry q); in this case, 3 equiv of TMG were used. The yields for 17 examples using the TMG method are compared with the best yields obtained under other conditions. In all but one case (entry c) the TMG methodology was proved to be superior or equal to the known methods.

In conclusion, 1,1,3,3-tetramethylguanidine (TMG) has been successfully applied in the S-alkylation of L-cysteine, D-penicillamine, and L-glutathione (with unprotected NH_2 and COOH groups) with various alkyl halides. Since no racemisation of the amino acids occurred during the reaction, the application of TMG can be considered a general route to activation of the SH group in amino acids and peptides.

Acknowledgments

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37. *Procedure for the synthesis of S-alkylated cysteine and related compounds* **3a–q**: A solution of TMG (21 mmol) in 5 ml of MeOH (or other alcohol) was added dropwise to a stirred (under argon) mixture of L-cysteine (10 mmol) in 10 ml of MeOH (or other alcohol). To the homogeneous solution was added alkylation reagent **2a–q** (10 mmol) in THF dropwise and the reaction mixture was stirred 1 h at 50 °C. The mixture was evaporated and after addition of 10 ml of H_2O, acidified with a stoichiometric quantity of AcOH or HCl (1:1). The separated precipitate was recrystallized from alcohol, dilute alcohol or H_2O—Method A. Method B: stoichiometric quantity of AcOH or HCl in MeOH (or EtOH) was added to the reaction mixture and more MeOH (or EtOH) to initiate crystallization of the product which was then recrystallized from alcohol or diluted alcohol.

Compounds **3a–q** are enantiomerically pure and were fully characterized on the basis of spectral (^{1}H, ^{13}C, IR) and CHN data.

Spectral data for new compounds: *compound* **3k**: from EtOH, mp 173–175 °C; $[\alpha]_D$ −5.6 (*c* 1.0, 0.1 M $NaOH_{aq}$); δ_H (400 MHz, D_2O) 3.97 (2H, dd, *J* = 3.2, 4.4), 3.28 (2H, dd, *J* = 14.8, 4.4), 3.15 (dd, 2H, *J* = 14.8, 7.6), 2.94 (4H, s); δ_C (100 MHz, D_2O) 173.61, 80.00, 54.04, 32.65, 19.89; ν_{max} (KBr/cm−1) 3410, 3045, 1607, 1408, 1131, 541 cm^{-1}; MS (EI) silylated derivative: $C_{22}H_{48}O_4N_2S_2Si_4$, *m*/*z* (%) = 581 (0.2) [M^+], 463 (18), 317 (19), 316 (14), 315 (7), 232 (9), 220 (11), 219 (22), 218 (100), 147 (13), 73 (24). Elemental anal.: $C_{10}H_{16}N_2O_4S_2$, calcd: C, 41.08; H, 5.52; N, 9.58; S, 21.93; obsd: C, 41.28; H, 5.41; N, 9.64; S, 22.14.

Compound **3m**: from methanol, mp 179–180 °C; $[\alpha]_D$ −23.62 (*c* 0.1, 0.1 M $NaOH_{aq}$); δ_H (400 MHz, NaOD/D_2O) 3.52 (1H, dd, *J* = 4.7, 7.5), 3.37 (1H, dd, *J* = 5.6, 7.5), 3.05 (1H, dd, *J* = 4.7, 13.5), 2.84 (1H, m, *J* = 5.6, 13.5), 2.83 (2H, s), 2.70 (1H, dd, *J* = 7.5, 13.5), 1.19 (9H, s,); δ_C (100 MHz, NaOD/D_2O) 181.57, 178.83, 70.34, 55.56, 40.08, 38.28, 30.21; ν_{max} (KBr/cm^{-1}) 3485, 3115, 2970, 1728, 1610, 1410, 1300, 1126, 675 cm^{-1}; MS (EI) silylated derivative: $C_{15}H_{33}O_4NS_2Si_4$, *m*/*z* (%) = 379 (0.02) [M^+], 234 (16), 190 (26), 146 (43), 134 (100), 116 (22), 75 (22), 73 (33), 57 (32), 43 (13); 28 (32). Elemental anal.: $C_9H_{17}NO_4S$, calcd: C, 45.94; H, 7.28; N, 5.95; S, 13.63; obsd: C, 46.01; H, 7.21; N, 5.98; S, 13.58.

Compound **3n**: from H_2O, mp 160–162 °C decomp. $[\alpha]_D$ 34.19 (*c* 0.1, 0.1 M $NaOH_{aq}$); δ_H (400 MHz, D_2O) 4.01 (1H, dd, *J* = 6.6, 5.6), 3.60 (2H, s), 3.28 (2H, m); δ_C (100 MHz, D_2O) 172.82, 116,67, 53.71, 33.21, 17.35; ν_{max} (KBr/cm^{-1}) 3426, 2976, 2247, 1587, 1509, 1397, 1344, 1300, 865, 546; MS (EI) silylated derivative: $C_{11}H_{24}N_2O_2SSi_2$, *m*/*z* (%) = 304 (0.14) [M^+], 219 (20), 218 (100), 188 (14), 187 (92), 147 (35), 146 (14), 144 (34), 100 (18), 75 (14), 73 (81). Elemental anal.: $C_5H_8N_2O_2S$, calcd: C, 37.49; H, 5.03; N, 17.49; S, 20.02; obsd: C, 37.52; H, 5.06; N, 17.53; S, 20.08.

Compound **3o**: from MeOH, mp 176–179 °C. $[\alpha]_D$ −85.11 (*c* 0.1, 0.1 M $NaOH_{aq}$), δ_H (400 MHz, NaOD/D_2O) 6.89 (4H, m), 6.46 (3H, m), 3.13 (2H, s), 2.97 (1H, s), 1.16 (3H, s), 0,84 (3H, s); δ_C (100 MHz, NaOD/D_2O) 178.71, 135.44, 133.53, 132.54, 128.58, 127.94, 127.71, 126.30, 125.90, 63.63, 51.33, 32.75, 27.71, 22.94; ν_{max} (KBr/cm^{-1}) 3504, 3151, 3046, 2966, 1622, 1508, 1410, 1370, 1132, 884, 836, 756, 564; MS (EI) silylated derivative: $C_{13}H_{28}N_2O_2SSi_2$, *m*/*z* (%) = 433 (0.08) [M^+], 220 (10); 219 (44), 218 (41), 215 (12), 142 (15), 141 (100), 115 (11), 73 (31), 32 (15), 28 (64). Elemental anal.: $C_{16}H_{19}NO_2S$, calcd: C, 66.41; H, 6.62; N, 4.84; S, 11.08; obsd: C, 66.59; H, 6.60; N, 4.78; S, 11.01.