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An improved method for cysteine alkylation

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Abstract—An improved method for the synthesis of *S*-alkylated cysteine derivatives with branched alkyl chains is reported. These compounds can be obtained in good yield and high purity by refluxing the cysteine thiol with the appropriate alkyl bromide in a solution of sodium ethoxide in ethanol. © 2001 Elsevier Science Ltd. All rights reserved.

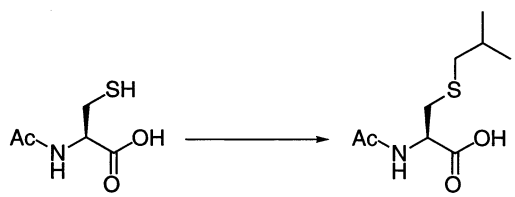
Posttranslational modification of cysteine residues by addition of an isoprenyl side chain to the sulfhydryl group is an important step for several proteins involved in signal transduction, such as yeast a-factor mating hormones, nuclear lamins, *ras* proteins and the γ-subunit of transducin. The majority of these alterations occur at a C-terminal cysteine with a 'CAAX' motif (C = cysteine, A = an aliphatic amino acid, X = any amino acid). In *ras* proteins, this modification allows the proteins to associate with the cellular membrane, a necessary step in the activation of these proteins for signal transduction.[1]

Kamiya et al. synthesized prenylated proteins via alkylation of the sulfhydryl group with farnesyl bromide in an alcohol/water mixture.[2] More recently, Yang et al. alkylated polypeptides in high yield by using an excess of farnesyl bromide in a mixed solvent system of DMSO/DMF/ACN containing diisopropylethylamine (DIEA).[3] Brown et al. prepared prenylated cysteine derivatives using farnesyl chloride in an ammonia/methanol solution.[4] A pentapeptide substrate, Lys-Cys-Val-Leu-Ser (which contains free amino, hydroxy and acid groups in addition to the cysteine thiol), was *S*-farnesylated using farnesyl chloride in refluxing liquid ammonia. Or et al. alkylated a model tetrapeptide, Ser-Leu-Cys-Phe, with a variety of alkyl substituents using the method[4] of Brown et al., demonstrating that this method is not restricted to allylic substrates.[5] Further, Xue et al. reported the regioselective isoprenylation of peptides in acidic conditions using zinc acetate as a catalyst.[6]

The anti-leukemia drug discovery research[7] undertaken in our laboratories has involved the synthesis of various *S*-alkylated cysteine derivatives. While our initial work in this area was successful using the method of Brown et al., attempts to synthesize 2-methylpropyl derivatives and other similarly β-branched aliphatic analogues failed. This prompted us to examine other conditions for cysteine alkylation. The results of this study and a comparison of various methods of cysteine alkylation are discussed in detail below.

The *S*-alkylation of *N*-acetyl-L-cysteine with 1-bromo-2-methylpropane was examined under various conditions (Table 1). The procedure developed by Brown et al. (Table 1, entry 1), which uses a solution of ammonia in methanol as the base, has been shown to selectively alkylate thiols even in the presence of other potential

Table 1. Comparison of various reaction conditions in the *S*-alkylation of *N*-acetyl-L-cysteine with 1-bromo-2-methylpropane



Entry	Method	Yield (%)
1	NH_3 in MeOH/16 h	~10[a]
2	Pyridine (cat. DMAP)/16 h	0
3	Et_3N in THF (cat. DMAP)/16 h	0
4	Et_3N in THF at reflux/4 h	0
5	NaOEt/EtOH at reflux/4 h	74
6	NaOMe/MeOH at reflux/4 h	81
7	NaH in THF/16 h	0
8	NaH in DMF/16 h	0

[a] Estimated by ^1H NMR, highly contaminated product.

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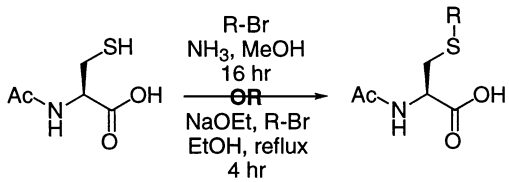
Table 2. Comparison of the yields obtained using the sodium ethoxide/ethanol versus the ammonia/methanol reaction conditions for the *S*-alkylation of various cysteine thiol derivatives with several different alkyl bromides



Entry	Thiol	Bromide	Yield	
			NaOEt	NH_3/MeOH
1	Acetyl-cysteine	(Bromomethyl)cyclohexane	73	0
2	Acetyl-cysteine	1-Bromo-2-ethylbutane	90	0
3	Acetyl-cysteine	1-Bromododecane	83	98
4	3-Mercaptopropionic acid	1-Bromododecane	68	21
5	Acetyl-cysteine	1-Bromo-2,2-dimethylpropane	28[a]	0
6	Acetyl-cysteine	2-Bromopropane	58	0
7	Acetyl-cysteine	Bromoethane	45	35
8	Acetyl-cysteine	*trans*, *trans*-Farnesyl bromide	96	99
9	Acetyl-cysteine	*trans*-Geranyl bromide	99	97
10	Cysteine methyl ester	1-Bromododecane	48	73

[a] Yield obtained after 12 hour reflux.

nucleophiles. In this case, however, the reaction did not proceed satisfactorily, giving poor yields of highly impure product. Using 1-iodo-2-methylpropane instead of its bromo counterpart did produce pure product in a 33% yield, although the lack of commercially available iodides does limit this approach. Most of the other methods provided no improvement and simply yielded starting material upon work-up. In contrast, the reactions which employed a sodium alkoxide (Table 1, entries 5 and 6) gave a pure product with no trace of either the alkyl bromide or the cysteine starting materials.[8] Additionally, the reaction involving sodium ethoxide produced a precipitate of NaBr, which indicated that the reaction was proceeding. This precipitate only formed while the reaction mixture was refluxing, however; at room temperature, no precipitate was produced and no appreciable reaction occurred.

Given the above results, the general utility of the method involving a sodium alkoxide was explored. Sodium ethoxide was selected for these studies rather than sodium methoxide since the formation of a precipitate when using the former provides a convenient visual indicator that the reaction is progressing. The reactions between *N*-acetyl cysteine and a variety of alkyl bromides were examined under these conditions (Table 2). Good yields were obtained for many of the bromides used, in several cases marking a substantial improvement over the results obtained using the literature method. In fact, for some of the alkyl bromides (e.g. 1-bromo-2-ethylbutane, Table 2, entry 2) the desired product was generated in excellent yield, whereas no product was obtained following the literature method. For the isoprenyl bromides (Table 2, entries 8 and 9), comparable yields were obtained using the new method and the literature procedure. However, there was little or no reaction between *N*-acetyl cysteine and 1-bromo-2,2-dimethylpropane under the new or the standard conditions; only after prolonged reflux was any significant material produced using the new method. This result suggests that while the method involving sodium ethoxide gives greatly improved yields for many branched alkyl bromides, reactions involving alkyl bromides that have a large amount of steric hindrance due to their highly branched nature (e.g. 1-bromo-2,2-dimethylpropane) are still inherently inefficient.

Two other thiols (Table 2, entries 4 and 10) were also studied to ascertain the effects of the amino group of cysteine (the amino group is absent in 3-mercaptopropionic acid, entry 4; cysteine methyl ester, entry 10, contains a free amino group) on the utility of the method involving sodium ethoxide. For the reaction of 3-mercaptopropionic acid with 1-bromododecane, an enormous improvement in yield was obtained using the new method (the yields using the method involving sodium ethoxide and the literature method of Brown et al. are 68 and 21%, respectively; Table 2). In contrast, the yield obtained for the reaction involving the cysteine methyl ester was reduced when using sodium ethoxide. These results suggest that while the method utilizing sodium ethoxide is clearly superior to its ammonia/methanol counterpart in some instances, in particular for reactions involving branched alkyl bromides, the reactions involving either a cysteine derivative bearing a free amino group or a less-hindered alkyl bromide (such as a straight-chain alkyl bromide or an isoprenyl bromide) benefit little from using this method.

One concern with using such relatively harsh basic conditions is the danger of racemization. Comparison of *N*-acetyl-S-dodecyl-L-cysteine samples made via the ammonia/methanol and sodium ethoxide methods

found identical optical rotations, indicating that no racemization had occurred. However, in the cysteine methyl ester case, this concern was well founded as the optical rotations obtained were widely different, which incidentally may account for the lower yield using this substrate.

An efficient reaction for the *S*-alkylation of cysteine provides a useful tool for exploring structure-activity relationships in cysteine-containing inhibitors and/or substrates. Elucidation of a variety of different experimental conditions under which this reaction can be performed is extremely useful for the synthesis of a wide variety of different compounds destined for biological testing. The method presented here provides a valuable complement to the methods already in the literature, giving access in particular to branched cysteine derivatives that are otherwise difficult to synthesize.

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8. A typical experiment was performed as follows: Freshly cut sodium metal (0.51 g, 22 mmol) was dissolved in anhydrous ethanol (50 mL) under an atmosphere of nitrogen. To the solution was added *N*-acetyl-L-cysteine (1.63 g, 10 mmol) followed by 1-bromo-2-methylpropane (1.51 g, 1.20 mL, 11 mmol) and the reaction mixture heated at reflux for 4 h, during which time a white precipitate was formed. Upon cooling, the reaction was quenched with a small amount of water, the solvent was removed under reduced pressure and the residue redissolved in ethyl acetate. The solution was washed with 1 M HCl and saturated brine, dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure to give the product, *N*-acetyl-*S*-2-methylpropyl-L-cysteine, as a white solid.