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Probing the isoprenylcysteine carboxyl methyltransferase (Icmt) binding pocket: Sulfonamide modified farnesyl cysteine (SMFC) analogs as Icmt inhibitors

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ABSTRACT

Human isoprenylcysteine carboxyl methyltransferase (hIcmt) is a promising anticancer target as it is important for the post-translational modification of oncogenic Ras proteins. We herein report the synthesis and biochemical activity of 41 farnesyl-cysteine based analogs versus hIcmt. We have demonstrated that the amide linkage of a hIcmt substrate can be replaced by a sulfonamide bond to achieve hIcmt inhibition. The most potent sulfonamide-modified farnesyl cysteine analog was **6ag** with an IC_{50} of 8.8 ± 0.5 μM for hIcmt.

Proteins that contain a C-terminal CaaX motif (where C-cysteine, aa-any aliphatic amino-acid, X being L, S, M, F or Q) are post-translationally modified by a series of reactions that enable their appropriate cellular targeting, most commonly membrane association (Fig. 1). K-Ras, one of the >120 proteins having a CaaX box undergoes farnesylation followed by endoproteolyic cleavage of the –aaX residues by Rce-1 and finally methyl esterification of the free carboxylate of the prenylated cysteine by isoprenylcysteine carboxyl methyltransferase (Icmt). Mutant K-Ras, stabilized in the constitutively active GTP-bound conformation, signals continuously resulting in tumorogenesis. Mutant Ras is implicated in 15–20% of all human malignancies and greater than 90% of pancreatic cancers.[1]

Inhibition of human Icmt (hIcmt), a 33 kDa integral ER membrane methyltransferase,[2] has been postulated to block the activity of oncogenic Ras. The validity of Icmt as a viable drug target was supported through a knock-out study performed by Bergo et al.[3] and later by Michaelson et al.[4] who showed that ablation of Icmt had a selective effect on Ras signaling. *N*-Acetyl-*S*-farnesyl-L-cysteine (AFC) is a substrate for human Icmt (hIcmt) and recent work in our laboratory has shown that amide modified farnesyl cysteine analogs (AMFCs) are low-micromolar inhibitors of hIcmt.[5,6] Other hIcmt inhibitors have also identified and vary in structure ranging from indoloacetamides,[7] thiosalicylic analogs[8] and prenylated thioacetic acid[9] to halogenated natural products.[10]

Due to its membrane localization and its lack of homology with other methyltransferases, there is no structural information on hIcmt. This poses a fundamental challenge to designing Icmt inhibitors. In an effort to understand the requirements for hIcmt inhibition, we hypothesized that isosteric replacements of the amide bond in AMFCs will result in molecules that will retain their interaction with hIcmt and provide more information on inhibition requirements. As a first step to realize this goal, we have focused our attention on evaluating metabolically stable amide bond replacements. Figure 2 shows the structure of the most potent AMFCs synthesized by Donelson et al.[6]

Replacement of the amide bond with a metabolically stable and more drug-like isostere is an important goal of our research. Its biological stability and the ease of synthesis led us to explore the sulfonamide bond in AFC based analogs. Herein, we demonstrate that the sulfonamide bond is a viable amide surrogate and that sulfonamide-modified farnesyl cysteine analogs (SMFCs) are low-micromolar inhibitors of hIcmt.

We synthesized 41 SMFCs through a facile two-step synthetic route from easily available starting materials as depicted in Scheme 1. L-Cysteine was *S*-farnesylated with farnesyl chloride in 7 N ammonia/methanol.[11] The resulting lipidated amino acid was coupled with various commercially available sulfonyl chlorides

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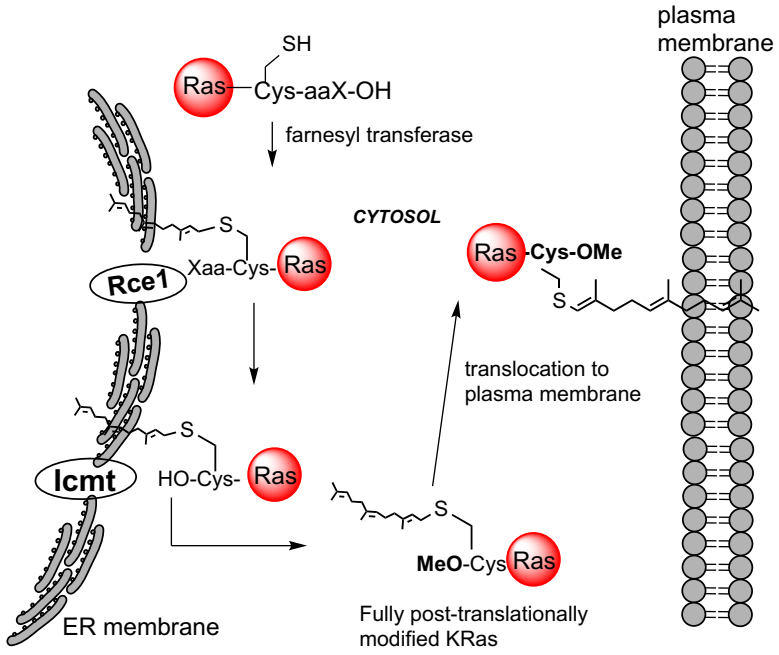


Figure 1. Post-translational modifications of Ras and transport from the ER to the plasma membrane.

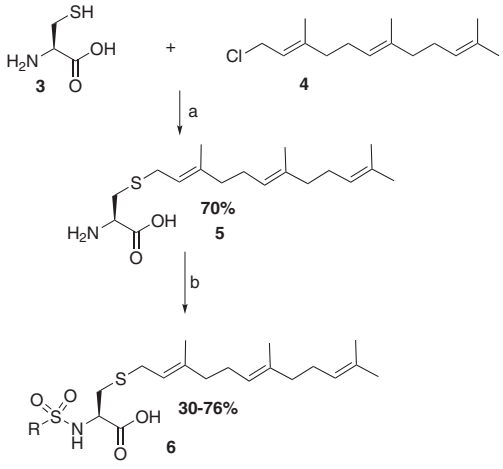
using aqueous sodium carbonate as the base and dioxane as solvent.

Our preliminary goal in using this SMFC library was to determine the structural requirements for hIcmt inhibition by prenylated cysteine derivatives containing the sulfonamide unit replacement. Chemically diverse sulfonyl chlorides were chosen for the synthesis of the SMFCs. Sulfonyl chlorides with different electronic character, steric bulk and alkyl/aromatic scaffolds were incorporated. All synthesized SMFCs were evaluated as substrates and inhibitors of human Icmt using the vapor diffusion assay (VDA).[12–14] The structures of the synthesized SMFCs are shown below in Figure 3.

The biochemical evaluation using the VDA revealed that no SMFCs are effective substrates, and are instead moderate inhibitors of hIcmt. The percent inhibition profiles of the SMFCs range from 22% to 60% hIcmt inhibition at 10 μM. Compounds **6a**–**q** inhibited human Icmt poorly, exhibiting less than 45% inhibition at 10 μM. All other analogs showed greater that 45% inhibition at the test concentration and their percent inhibition values are listed in Figure 3. Compounds **6r**–**ab** inhibited hIcmt between 45% and 55% at 10 μM and compounds **6ac**–**ag** inhibited hIcmt more than 55% at 10 μM.

IC_{50} determinations were carried out for a select group of compounds **6r**, **6s**, **6ae**, **6af** and **6ag**. Given below in Table 1 are the IC_{50} values for the selected compounds.

Our data establish that all SMFCs are relatively poor to moderate inhibitors of hIcmt. Surprisingly, none of the SMFCs are substrates for hIcmt (data not shown). We hypothesize that the presence of the amide bond, or a carbonyl carbon may be necessary for molecules to exhibit substrate activity. All SMFCs inhibit hIcmt, but vary in the strength of inhibition. Highly bulky or rigid analogs (**6a**, **b** and **h**) are poor hIcmt inhibitors, although **6ab** and **6y** are



Scheme 1. Reagents and conditions: (a) 7 N NH_3/MeOH, 0 °C – rt, overnight; (b) 10% Na_2CO_3/dioxane (1:1), then RSO_2Cl, rt, overnight.

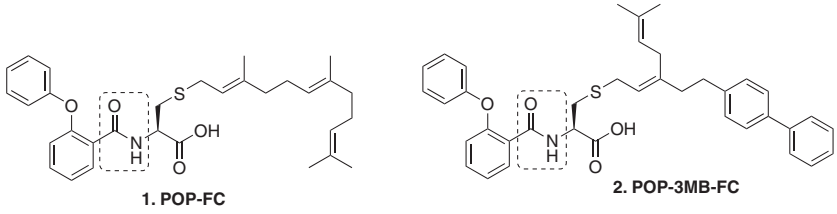


Figure 2. Phenoxyphenyl-farnesyl cysteine (POP-FC) and phenoxy-phenyl-3-methylbutenyl-biphenyl farnesyl cysteine (POP-3MB-FC).[6] The metabolically labile amide bond is highlighted.

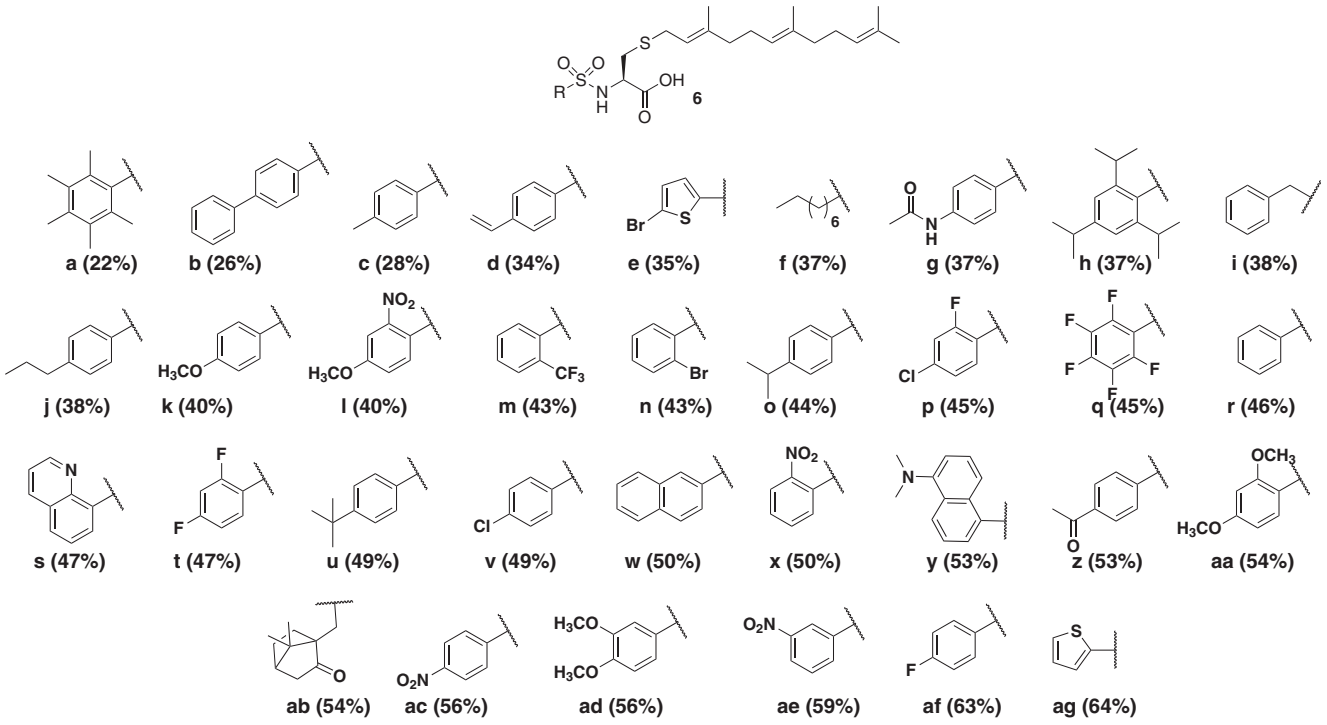


Figure 3. Structures of the sulfonamide-modified farnesyl cysteine (SMFC) analogs synthesized. The numbers in the parentheses indicate the percent inhibition by the SMFC analog in the vapor diffusion assay at 10 μM using 25 μM AFC as the substrate.

Table 1
IC_{50} values of selected SMFCs

Compound	IC_{50}[a] (μM)
6r	17.0 ± 1.6
6y	11.6 ± 1.3
6ag	8.8 ± 0.5
6ae	13.4 ± 1.5
6af	15.5 ± 1.8
–	–

[a] IC_{50} values are mean of three experiments, were determined using the VDA and calculated using GRAPHPAD PRISM 5.0. Concentration of the substrate used (AFC) was 25 μM.

Table 2
Percent inhibition values for prenyl modified SMFCs

Compound	Percent inhibition[a]
7a	22
7b	18
7c	23
8a	17
8b	32
–	–

[a] Percent hIcmt inhibition at 10 μM inhibitor concentration. AFC is used as a normalizing control and specific activities are converted to percent inhibition.

exceptions. We determined that compounds containing electron withdrawing groups were generally better as hIcmt inhibitors as compared to the ones with electron donating functionalities (**6af**, **ae**, **ac** vs **6c**, **j**, **k**, **l**); although there are exceptions (e.g., **6p** and **6q**). Planar bulk next to the sulfonamide bond also enhances inhibition (**6s**, **w** and **y**).

We next wanted to evaluate the effect of the prenyl group in SMFCs on hIcmt inhibition. To achieve this goal, we synthesized three sulfonamide-modified geranyl cysteine (SMGC) analogs and two analogs where the prenyl chain was replaced by an alkyl chain. These analogs were synthesized in a similar manner to the SMFCs.

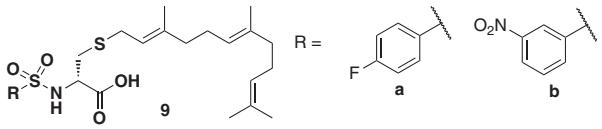


Figure 5. Structures of D-SMFCs synthesized.

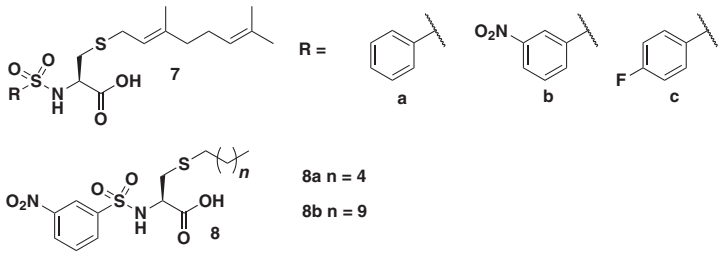
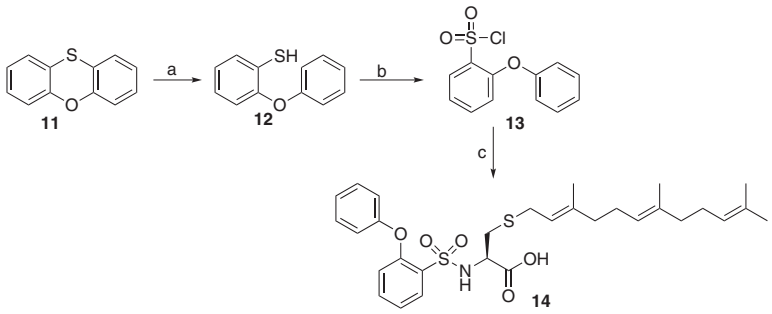


Figure 4. Structures of prenyl-modified SMFCs.



Scheme 2. Reagents and conditions: (a) Li wire, DTTB, THF, −78 °C to room temp, 45%; (b) NCS (4 equiv), 2 M HCl/ACN (1:1), 20 °C, 92%; (c) farnesyl cysteine, 2N Na_2CO_3/dioxane, 63%.

For the analogs that contain the alkyl chain, the base in the *S*-alkylation step was changed to 2 M sodium hydroxide in ethanol as described before (see Supplementary data for more detail).[15,16] Although racemization at the alpha carbon is certainly a possibility, we did not investigate for this possibility. The structures of these analogs are shown in Figure 4. These analogs were evaluated as inhibitors and substrates of hIcmt using the VDA.

The percent inhibition of compounds **7a**–**c** and **8a**–**b** are shown in Table 2. None of these compounds exhibited substrate activity at 25 μM.

Analogs **7a**–**c** and **8a**–**b** are relatively poor inhibitors of hIcmt as compared to their farnesyl analogs (compounds **6r**, **6ae** and **6af**). These data illustrate that the farnesyl group is highly important for hIcmt inhibition by SMFCs. Increased chain length of the prenyl group appears to be a key factor in hIcmt inhibition. Also, the fact that analog **8a** exhibits very similar inhibition to **7b** illustrates that the geranyl and the hexyl chains make little difference in the biological activities of these analogs. The fact that compound **8b** only showed marginal improvement in inhibition over **8a** also strengthens our hypothesis that a longer prenyl chain is a key for hIcmt inhibition. Overall, these data suggest that a determining factor for hIcmt inhibition by SMFCs is the presence of the farnesyl chain, imparting a specific binding interaction rather than simply hydrophobic bulk.

Having determined the role of the farnesyl group in hIcmt inhibition by SMFCs, we next wanted to evaluate the effect of stereochemistry at the alpha carbon of the SMFCs. To achieve this goal, we synthesized the (*S*) enantiomers of compounds **6ae** and **6af**. These were synthesized in a manner similar to the one shown in Scheme 1, using D-cysteine in place of L-cysteine. The structures of these analogs are shown in Figure 5.

Interestingly, analogs **9a**–**b** exhibited identical inhibition profiles at 10 μM as compared to compounds **6ae** and **6af**. There was little to no difference in the inhibition profiles of the two enantiomers (data not shown). The enantiomers also did not exhibit any substrate activity. The fact that stereochemistry at the alpha carbon did not play a significant role in hIcmt inhibition by SMFCs coupled with the knowledge that the farnesyl group appeared to be a key factor for inhibition leads us to hypothesize that the farnesyl chain in either enantiomer is able to adopt a favorable conformation and enables the SMFC to inhibit hIcmt.

Next, we wanted to evaluate the importance of the carboxylate motif of SMFCs for hIcmt inhibition. Toward this aim, we synthesized the methyl ester analog (compound **10**) of **6ag** (details for synthesis in Supplementary data). Strikingly, this analog exhibited only 10% hIcmt inhibition at 10 μM in the VDA. Although compound **6ag** is the most potent SMFC, its ester, compound **10**, is a remarkably poor hIcmt inhibitor. This leads us to hypothesize that a deviation from the carboxylate group greatly reduces hIcmt inhibitory activity although more analogs need to be evaluated to corroborate this result.

Finally, we wanted to incorporate the phenoxyphenyl motif (Fig. 2) that is a part of the most potent AMFCs into the SMFC scaffold. As 2-phenoxy-phenyl sulfonyl chloride is not commercially available, we synthesized it and then coupled it to farnesyl cysteine. Scheme 2 shows the synthesis of POP-SMFC that uses a lithium mediated homolytic C–S bond cleavage in phenoxathin[17] followed by oxidative chlorination[18] to yield the sulfonylchloride **13** of interest.

Compound **14**, the SMFC analog of POP-FC (**1**) inhibits hIcmt by 55% at 10 μM and has an IC_{50} of 18.4 ± 1.8 μM as determined by the VDA. Although most SMFCs are better, or equal inhibitors of hIcmt as compared to AMFCs, POP-SMFC (**14**) is a poorer inhibitor of hIcmt as compared to its amide counterpart.

Overall, we have shown that the sulfonamide bond is a viable replacement for the amide bond in AFC derived hIcmt inhibitors. We have also explored the structure–activity relationship of SMFCs as hIcmt inhibitors. The most potent analog, **6ag** has an IC_{50} of 8.8 ± 0.5 μM. We have demonstrated that the farnesyl group of SMFCs is a necessary structural motif for hIcmt inhibition and that stereochemistry of the alpha carbon does not play a significant role in inhibiting hIcmt. We have also highlighted the importance of the carboxylate motif in SMFCs for hIcmt inhibition, but this result needs further evaluation.

In comparison to the AMFCs, the SMFC library is superior for a number of reasons. Notably, the lead SMFC **6ag** exhibits superior ligand efficiency[19] to POP and POP-3-MB. While the AMFC library yielded several substrates for hIcmt, the SMFC library showed no substrate activity. Our lead, **6ag** is a step in the right direction because it has a lower molecular weight, a lower *C* Log *P*, a higher tPSA and overall a more 'drug-like' character. These encouraging data are fueling our efforts in designing and evaluating novel scaffolds as amide isosteres for achieving potent hIcmt inhibition.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.078.

References and notes

1. Konstantinopoulos, P. A.; Karamouzis, M. V.; Papavassiliou, A. G. *Nat. Rev. Drug Disc.* **2007**, *6*, 540.

2. Dai, Q.; Choy, E.; Chiu, V.; Romano, J.; Slivka, S. R.; Steitz, S. A.; Michaelis, S.; Philips, M. R. *J. Biol. Chem.* **1998**, *273*, 15030.
3. Bergo, M. O.; Leung, G. K.; Ambroziak, P.; Otto, J. C.; Casey, P. J.; Gomes, A. Q.; Seabra, M. C.; Young, S. G. *J. Biol. Chem.* **2001**, *276*, 5841.
4. Michaelson, D.; Ali, W.; Chiu, V. K.; Bergo, M.; Silletti, J.; Wright, L.; Young, S. G.; Philips, M. *Mol. Biol. Cell* **2005**, *16*, 1606.
5. Donelson, J. L.; Hodges, H. B.; MacDougall, D. D.; Henriksen, B. S.; Hrycyna, C. A.; Gibbs, R. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4420.
6. Donelson, J. L.; Hodges-Loaiza, H. B.; Henriksen, B. H.; Hrycyna, C. A.; Gibbs, R. A. *J. Org. Chem.* **2009**, *74*, 2975.
7. Winter-Vann, A. M.; Baron, R. A.; Wong, W. H.; Dela Cruz, J.; York, J. D.; Gooden, D. M.; Bergo, M. O.; Young, S. G.; Toone, E. J.; Casey, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4336.
8. Gana-Weisz, M.; Paz, A.; Haklai, R.; Marciano, D.; Kloog, Y. *Neurosci. Lett.* **1997**, S17.
9. Tan, E. W.; Perezsala, D.; Canada, F. J.; Rando, R. R. *J. Biol. Chem.* **1991**, *266*, 10719.
10. Buchanan, M. S.; Carroll, A. R.; Fechner, G. A.; Boyle, A.; Simpson, M. M.; Addepalli, R.; Avery, V. M.; Hooper, J. N. A.; Su, N.; Chen, H. W.; Quinn, R. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6860.
11. Brown, M. J.; Milano, P. D.; Lever, D. C.; Epstein, W. W.; Poulter, C. D. *J. Am. Chem. Soc.* **1991**, *113*, 3176.
12. Hrycyna, C. A.; Yang, M. C.; Clarke, S. *Biochemistry* **1994**, *33*, 9806.
13. Hrycyna, C. A.; Clarke, S. *Mol. Cell. Biol.* **1990**, *10*, 5071.
14. Anderson, J. L.; Frase, H.; Michaelis, S.; Hrycyna, C. A. *J. Biol. Chem.* **2005**, *280*, 7336. Brief procedure for the in vitro methyltransferase vapor diffusion assay: Membrane protein (5 μg) was added to a solution of Tris–HCl buffer (100 mM, pH 7.4) and AFC (200 μM). After 5 min incubation on ice, 20 μL of *S*-adenosyl-L-[*methyl*-^{14}C]methionine ([^{14}C]SAM) (50–60 mCi/mmol) (60 μM) is added and the solution is incubated at 30 °C in a water bath for 30 min. After 30 min the reaction is stopped by the addition 50 μL of 1 M NaOH/1% SDS. The reaction mixture is vortexed and spotted on to a pleated filter paper. The filter paper is lodged into the neck of a scintillation vial filled with 10 mL of scintillation fluid and capped. The filter papers were removed after 2.5 h and the radioactivity was measured using a Packard 1600CA Liquid Scintillation Analyzer..
15. Armstrong, M. D.; Lewis, J. D. *J. Org. Chem.* **1951**, *16*, 749. About racemization at the alpha carbon, this Letter states that, 'Some concern was felt when it was noted that many of the derivatives of cysteine had very small numerical values for their rotations when they were measured in acid solution, since derivatives of cysteine and serine are known to racemize easily in alkaline solution. Measurement of the rotations of these compounds in water or in alkaline solutions gave much larger numerical rotations, however, and it was firmly established that, once freed of cystine, no further change in their rotation could be observed after repeated treatments with ammonia and sodium cyanide.'.
16. Frankel, M.; Gertner, D.; Jacobson, H.; Zilkha, A. *J. Chem. Soc.* **1960**, 1390.
17. Yus, M.; Foubelo, F.; Ferrandez, J. V. *Chem. Lett.* **2002**, 726.
18. Nishiguchi, A.; Maeda, K.; Miki, S. *Synthesis-Stuttgart* **2006**, 4131.
19. Reynolds, C. H.; Bembenek, S. D.; Tounge, B. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4258.