# Solid-phase synthesis of tri-functionalised, α-substituted carbamoylmethyl-homocysteine compounds, their release from the resin and subsequent intramolecular cyclisation to give novel 1,3,3-trisubstituted succinimides

**J. Alan Girdwood and Richard E. Shute***

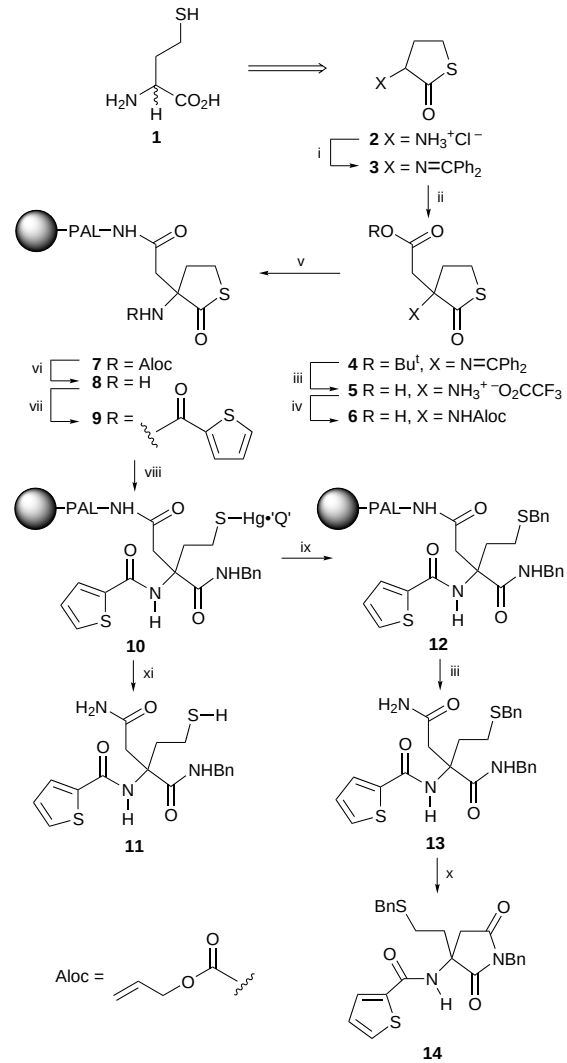
*ZENECA Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire, UK SK10 4TG*

**Attachment of DL-homocysteine thiolactone to polystyrene resin *via* the PAL-linker and an α-carbamoylmethyl group, followed by amino, carboxy and thiol derivatisation, including a novel Hg-assisted thiolactone ring-opening, and acidolytic cleavage from the solid-phase yields novel 1,3,3-trisubstituted succinimides *via* intramolecular cyclisation.**

Recently, the pharmaceutical industry has undertaken far-reaching changes in order to foreshorten the timelines of drug discovery and development. Within the former of these two processes, the technology of combinatorial chemistry has assumed an ever-increasing importance.[1] Fundamental to the recent development of this technique has been the use of solid-phase organic chemistry (SPOC).[2] Here we report: (i) attachment of the amino acid homocysteine (hCys) **1** to a polystyrene-based solid-support *via* the PAL-linker[3] and an acetate-derived spacer group attached to the α-carbon of the amino acid; (ii) on-resin derivatisation of the orthogonal carboxy, amino and thiol functionalities; (iii) release of target compounds from the resin; and (iv) an unexpected intramolecular cyclisation of the target compounds leading to novel 1,3,3-trisubstituted succinimide compounds. Derivatisation of the amino functionality was achieved by means of a novel heavy metal-assisted ring-opening of the thiolactone. The chemistry described has been optimised to take place under mild conditions, to be high yielding, to use diverse substituents or building blocks which are readily available, and to yield compounds of high purity. It is thus eminently amenable to the generation of a compound library suitable for general biological screening purposes.

With the proviso that we wished to preserve the three hCys functionality handles for on-resin derivatisation, we envisaged connection of DL-hCys thiolactone **2**, a cheap and readily available precursor to hCys, to the resin *via* an acidolytic amide linker group attached to the α-carbon (Scheme 1). Off-resin reaction of **2** with benzophenone imine yielded the stable Schiff's base **3**.† Deprotonation of the α-position by KOBu$^t$ was followed by alkylation with $BrCH_2CO_2Bu^t$ to give **4** in good yield. Treatment of **4** with dilute acid gave the thiolactone amino acid **5** which could be readily $N^{\alpha}$-protected by treatment with allyl chloroformate (Aloc-Cl) under Schotten–Baumann conditions to give the crystalline, protected template **6**. Complete attachment of **6** to PAL-polystyrene resin[3] to give **7** was achieved by means of standard coupling reagents and a two-fold excess of **6**. $Pd^0$-catalysed Aloc-deprotection of **7** was achieved within 2 h at room temp. as indicated by cessation of $CO_2$ evolution, to give **8**. Coupling of the model carboxylic acid thiophene-2-carboxylic acid to **8** was achieved using an excess of symmetrical anhydride and yielded **9**.§

Attempts to ring-open the thiolactone of **9** to give amide and free thiol by treatment with $BnNH_2$ in *N*-methylpyrrolidone at elevated temperatures over many hours were unsuccessful. This was indicated by our failure, not only to observe the loss of the diagnostic thiolactone carbonyl absorption peak at *ca*. 1700 $cm^{-1}$ in the FT-IR spectrum of resin **9** (data gathered on a mull of **9** in $CHCl_3$), but also, on cleavage and analysis by HPLC and mass spectrometry, to see any compound other than that based on starting material. Addition of catalysts such as DMAP or NaCN also failed to effect the desired reaction. Finally, on-resin ring-opening was achieved by means of a novel Hg-assisted reaction in the presence of $BnNH_2$ to yield **10**.¶ The exact nature of the resultant resin-bound thiolate species, –S–Hg–'Q', remains unclear. Acidolytic cleavage of **10** using TFA (aq.) and




**Scheme 1** *Reagents and conditions*: i, $Ph_2C{=}NH$, $CH_2Cl_2$, room temp., 91%; ii, KOBu$^t$, THF, $BrCH_2CO_2Bu^t$, room temp., 89%; iii, TFA (aq.), 90%; iv, $CH_2{=}CHCH_2O_2CCl$, dioxane, $Na_2CO_3$ (aq.), room temp., 75%; v, PAL-resin, $Pr^iN{=}C{=}NPr^i$, 1-hydroxybenzotriazole, DMF, room temp. vi, $Pd(PPh_3)_4$, $Bu^n{}_3SnH$, AcOH, $CH_2Cl_2$, room temp. vii, thiophene-2-carboxylic anhydride, DMF, pyridine, room temp.; viii, $BnNH_2$, $Hg(O_2CCF_3)_2$, THF, room temp.; ix, BnBr, THF, room temp.; x, prolonged treatment with TFA–$H_2O$ 9 : 1, room temp. or 1% conc. HCl in MeOH, room temp., 90%; xi, TFA, $Et_3SiH$ 99 : 1

examination of the product by HPLC and mass spectrometry yielded material that contained 2–3 distinct chemical species, none of which was readily characterisable. On the other hand, when TFA–$Et_3SiH$ was used instead of TFA (aq.), the free thiol, **11**, as characterised by HPLC and mass spectrometry, was produced in high yield and purity. Elemental analysis of resin **10** showed Hg and F to be present as well as the other expected elements, suggesting that 'Q' comprised a $CF_3CO_2^-$ counterion derived from $Hg(O_2CCF_3)_2$. However, closer analysis of the elemental composition revealed a F : S ratio significantly less than 3 : 1. This further implied that 'Q' was not wholly $CF_3CO_2^-$ anion, but rather suggested a mixture of $CF_3CO_2^-$ and a putative crosslinked –S–Hg–S– form with two neighbouring on-resin hCys thiolates joined through a mercuric ion. Such a mixture of on-resin species might explain the observation of a mixture of products on cleavage of **10** under non-reducing conditions.

The final stage in the on-resin synthesis was achieved by direct treatment of **10** with excess BnBr to give **12**.**

Treatment of **12** with TFA (aq.) for 1 h gave **13,**‖ the conformationally flexible hCys product that was our initial target, in over 60% overall yield and with a purity greater than 90% by HPLC. Somewhat unexpectedly however, if the acid treatment time was increased, we observed in the reversed-phase HPLC a later running impurity, which, under prolonged TFA (aq.) treatment, became the predominant, and most stable chemical species. HPLC and mass spectrometry indicated that this material was derived from **13** by loss of $NH_3$. Furthermore, treatment of chromatographically purified **13** with HCl in MeOH (aq.) rapidly gave **14**‖ in greater than 90% yield. This material was identical to the later running impurity observed on prolonged treatment of **13** with TFA (aq.).

Compound **14** arises *via* an intramolecular cyclisation analogous to the peptidyl 'aspartimide' reaction, a rearrangement which can, in Asx-Gly/Ser containing peptides, lead to unnatural β-linked isoAsx-Gly/Ser sequences.[5] This cyclisation has a number of major consequences for the chemistry as originally conceived. Firstly, it removes the primary carboxamide functionality, that vestige of the compound's link to the solid support, by tying it back to the rest of the molecule in a succinimide ring. Secondly, and perhaps of greater significance in the context of this chemistry being of utility for the generation of compound libraries, the cyclisation does not cause any of the introduced diversity substituents to be lost. The final product **14** still retains all the functionality attached to hCys as was required in the original design, only now displayed around a different, more constrained scaffold.

Finally, it is of note that substituted succinimides have a long and rich history in medicinal chemistry particularly as bioisosteres of hydantoins,[6] and also that the hydantoin ring system itself was not only one of the very earliest heterocycles to be exploited in combinatorial chemistry,[7] but continues to be of interest for the generation of diverse compound libraries.[8] In a similar fashion, 1,3,3-trisubstituted succinimide-based compound libraries†† derived, as we have shown, either from α-carbamoylmethylhomocysteine, or potentially from other α-carbamoylmethyl-substituted amino acids, should also be of utility in drug discovery programmes. Our further studies on compounds of this general type[10] will be reported in due course.

The authors acknowledge the contributions of Peter Robins, Rachel Stead, Dearg Brown, Brian Main, David Hollinshead, Brian Wright, Kath Barber and Eddie Clayton to this work.

## Footnotes and References

* E-mail: richard.r.e.shute@alderley.zeneca.com

† For a recent reference to the solution-phase chemistry of amino acid Schiff's bases of this type see ref. 4(*a*). For a recent reference to on-resin, amino acid Schiff's base chemistry see ref. 4(*b*).

§ In order to confirm the integrity and follow the progress of any particular reaction, acidolytic cleavage of a few milligrams of resin was performed at various times throughout the course of all reactions by treatment with TFA in the presence of either $Et_3SiH$ or $H_2O$ and the products examined by HPLC and mass spectrometry. Also, all intermediate resins were analysed by quantitative elemental analysis for changes in heavy atom percentages as a means of estimating on-resin chemical yields.

¶ Further investigation of the scope of this reaction indicated that $Hg(O_2CCF_3)_2$ (2–3 equiv.) in the presence of excess amine was required for efficient and high yielding reaction, and also that only primary aliphatic amines were effective.

‖ *Selected physical data* for: **13**: $\delta_H([^2H_6]DMSO)$: 2.30 (m, 4 H), 2.96 (ABq, 2 H), 3.62 (s, 2 H), 4.30 (d, 2 H), 6.86 (br, 1 H), 7.23 (m, 12 H), 7.16 (m, 1 H), 7.27 (m, 1 H), 8.28 (s, 1 H), 8.49 (br, 1 H); $\nu_{max}(CHCl_3)/cm^{-1}$ 1667, 1638 (C=O). For **14**: $\delta_H([^2H_6]DMSO)$ 2.10 (m, 2 H), 2.42 (m, 2 H), 2.93 (ABq, 2 H), 3.72 (s, 2 H), 4.59 (ABq, 2 H), 7.16 (m, 1 H), 7.29 (m, 10 H), 7.78 (m, 1 H), 7.92 (m, 1 H), 8.80 (s, 1 H); $\nu_{max}(CHCl_3)/cm^{-1}$ 1777, 1708, 1619 (C=O).

** Extensive washing of the resin at this stage with solvents (THF, $CH_2Cl_2$, MeOH and $Et_2O$) and with dithiothreitol in THF gave material which, under stringent elemental analysis conditions, showed no evidence of any residual mercury (not greater than 1 ppm).

†† During the course of our development work on this chemistry we used the following nine diversity reagents: AcOH, thiophene-2-carboxylic acid, 2-naphthyloxyacetic acid (≡'A'–$CO_2H$); $BnNH_2$, isoamylamine, methoxyethylamine (≡'B'–$NH_2$); bromophenacyl bromide, 4-methoxybenzyl chloride and 4-nitrobenzyl bromide (≡'C'–$CH_2Br$), in the parallel synthesis of a $3 \times 3 \times 3$, 27-membered combinatorial library of discrete, single compounds. HPLC chromatography, with UV and evaporative light scattering detection (ref. 9), as well as mass spectrometry analyses of the 27 final products indicated that in all cases the correct succinimide products, which are analogues of **14**, were formed in greater than 50% overall yield and in greater than 70% purity. (Analytical data for these 27 succinimide compounds are available from the authors.)

1 M. A. Gallop, R. W. Barrett, W. J. Dower, S. P. A. Fodor and E. M. Gordon, *J. Med. Chem.*, 1994, **37**, 1233; E. M. Gordon, R. W. Barrett, W. J. Dower, S. P. A. Fodor and M. A. Gallop, *J. Med. Chem.*, 1994, **37**, 1385; N. K. Terrett, M. Gardner, D. W. Gordon, R. J. Kobylecki and J. Steele, *Tetrahedron*, 1995, **51**, 8135; G. Lowe, *Chem. Soc. Rev.*, 1995, **24**, 309; J. A. Ellman, *Acc. Chem. Res.*, 1996, **29**, 132; D. V. Patel and E. M. Gordon, *Drug Discovery Today*, 1996, **1**, 134.

2 For recent reviews, see: J. S. Fruchtel and G. Jung, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 17; P. H. H. Hermkens, H. C. J. Ottenheijm and D. Rees, *Tetrahedron*, 1996, 5**2**, 4527; L. A. Thompson and J. A. Ellman, *Chem. Rev.*, 1996, **96**, 555; P. H. H. Hermkens, H. C. J. Ottenheijm and D. Rees, *Tetrahedron*, 1997, **53**, 5643.

3 F. Albericio, N. Kneib-Cordonier, S. Biancalana, L. Gera, R. I. Masada, D. Hudson and G. Barany, *J. Org. Chem.*, 1990, **55**, 3730.

4 (*a*) A. Lopez, M. Morenomanas, R. Pleixats, A. Roglans, J. Ezquerra and C. Pedregal, *Tetrahedron*, 1996, **52**, 8365; (*b*) M. J. O'Donnell, C. Zhou and W. L. Scott, *J. Am. Chem. Soc.*, 1996, **118**, 6070.

5 R. W. Roeske, in *The Peptides: Analysis Synthesis and Biology*, ed. E. Gross and J. Meienhofer, Academic Press, New York, 1981, vol. 3, pp. 102–136.

6 *Comprehensive Medicinal Chemistry*, ed. C. Hansch, P. G. Sammes and J. B. Taylor, Pergamon Press, Oxford, 1990, vol. 1, p. 55.

7 S. H. DeWitt, J. S. Kiely, C. J. Stankovic, M. C. Schroeder, D. M. Reynolds Cody and M. R. Pavia, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 6909.

8 B. A. Dressman, L. A. Spangle and S. W. Kaldor, *Tetrahedron Lett.*, 1996, **37**, 937; S. Hanessian and R.-Y. Yang, *Tetrahedron Lett.*, 1996, **37**, 5835.

9 M. Dreux, M. Lafosse and L. Morin-Allory, *LC GC Int.*, 1996, 148; C. Kibbey, *Mol. Diversity*, 1996, **1**, 247.

10 For recent references to similar 1,3,3-trisubstituted succinimides, see: W. L. Scott, C. Zhou, Z. Fang and M. J. O'Donnell, *Tetrahedron Lett.*, 1997, **38**, 3695; A. D. Abell and M. D. Oldham, *J. Org. Chem.*, 1997, **62**, 1509; D. Obrecht, C. Abrecht, M. Altorfer, U. Bohdal, A. Grieder, M. Kleber, P. Pfyffer and K. Mueller, *Helv. Chim. Acta*, 1996, **79**, 1315; M. L. Pedersen and D. B. Berkowitz, *J. Org. Chem.*, 1993, **58**, 6966; N. Sewald, J. Riede, P. Bissinger and K. Burger, *J. Chem. Soc., Perkin Trans. 1*, 1992, 267.

*Received in Glasgow, UK, 17th July 1997; 7/05757A*