Aminoacid-derived mercaptoimidazoles†

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Starting from suitably protected aminoacids, mercaptoimidazoles were synthesized either from the acid or including the amine nitrogen itself. A preliminary optimisation study led to efficient conditions for the obtention of the imidazole ring. These conditions are compatible with the presence of aminoacid or dipeptide scaffolds.

Introduction

Peptidomimetics[1] have been thoroughly studied for decades as aminoacid or peptide surrogates. Their main requirements in terms of chemical properties consist of a structural mimicry of natural peptides, though avoiding their shortcomings. Thus, the amide bond surrogate should be stable towards enzymatic hydrolysis, display a low toxicity and/or good bioavailability. Most peptidomimetics either feature a replacement of the scissile amide bond itself for instance with azapeptides[2] or fluoroolefins,[3] or incorporate aminoacid side-chains into constrained structures such as azabicycloalkanes of various sizes.[4] In this work, we focused our attention towards the incorporation of the *N*-terminus amine or *C*-terminus acid of aminoacids into a rigid, aromatic heterocyclic scaffold (Fig. 1). In view to explore new series of potential zinc exopeptidases inhibitors, the 4-mercaptoimidazole scaffold was chosen as a binding motif for the metal.

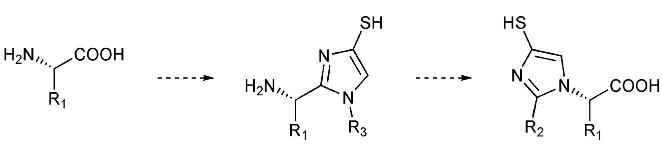

Fig. 1 *N*- or *C*-terminus aminoacid-derived mercaptoimidazoles.

In the past thirty years,[5] thiol inhibitors have been widely developed. The imidazole ring which is found in histidine often accounts for the chelation of the zinc cation in the binding site of the enzyme.[6] In addition, mercaptoimidazoles are appealing targets for their antioxidant properties. This moiety is present in natural compounds such as ergothioneine or ovothiol.[7] In this paper, we wish to report a validation of a general method for the conversion of aminoacids into their mercaptoimidazole analogs.

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Results and discussion

As far as the synthesis of 4-mercaptoimidazoles is concerned, few procedures exist. Condensation can be achieved either from an alkylsulfanyl enamine using propane phosphonic anhydride[8] or from *N*-acyl, *N*-alkylaminothioacetamide, readily available from aminoacetonitrile precursors (Fig. 2). The cyclization step requires a selective electrophilic activation of the carboxamide, over the thioamide. This can be achieved if the dehydrating reagent has a better affinity for oxygen than nitrogen or sulfur. Previously, Hopkins *et al.*[9] used trimethylsilyl triflate. This procedure, which was successfully employed later,[10] and is amenable to selenoimidazoles,[11] is very efficient and allows smooth formation of 4-mercaptoimidazoles. Further workup of the reaction requires the use of $NaBH_4$ to avoid obtaining disulfides.

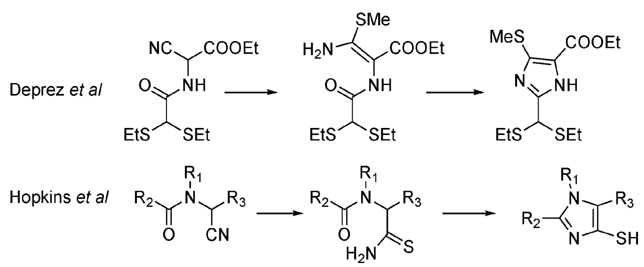


Fig. 2 Strategies described in the literature.

In the case of aminoacids and peptides, we chose (Fig. 3) the method described in ref. 9, believing that those mild conditions would be suitable with highly-functionalized aminoacid derivatives. Preliminary results, however, called for further optimisation of the cyclisation step and the introduction of a subsequent protection step.

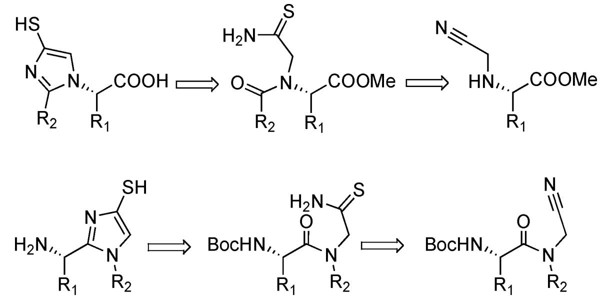
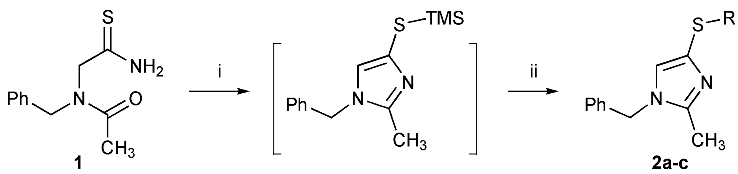


Fig. 3 Retrosynthetic analysis.



Scheme 1 *Reagents and conditions:* i, TMSOTf, Et_3N, CH_2Cl_2, –78 °C to RT, 6.5 h; ii, see conditions in Table 1.

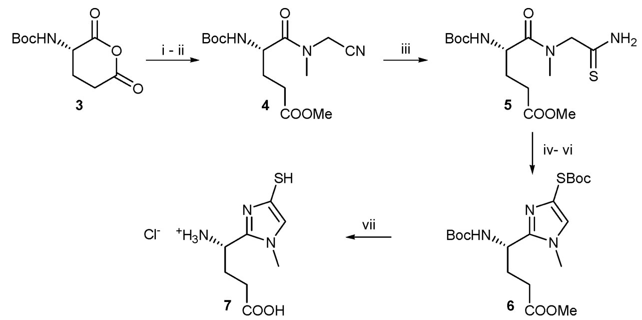
Unraveling the reactivity of this system was initially conducted with *N*-acetyl-*N*-benzylaminothioacetamide **1** as a model substrate before being applied to aminoacids. As shown in Scheme 1, several parameters were examined to reach an efficient one-pot process. Preparation of the reagent was accomplished by treating *N*-benzyl aminoacetonitrile with excess thioacetic acid in pyridine[12] followed by acetylation. Dehydration of this compound with TMSOTf/Et_3N following the literature conditions led to a modest yield of 4-mercaptoimidazole.

In addition, the imidazole thiol may be isolated as a thiol or disulfide but characterisation of this compound is facilitated by protecting the thiol moiety. On the one hand, pK_a values[13] indicate that 4-mercaptoimidazoles predominantly exist under a zwitterionic form, since 1,5-dimethyl-4-mercaptoimidazole exhibited a pK_a of 2.3 for the thiol and 10.3 for the imidazole itself. On the other hand, as free thiols, mercaptoimidazoles in particular are very potent anti-oxidants, and they have a high propensity to undergo oxidative dimerisation. Their corresponding disulfides were more commonly isolated. Thus, *in situ* electrophilic trapping was used as a mean to facilitate the isolation of the products. As listed in Table 1, *in situ* protection of the thiol was performed with benzylic halides or di-*tert*-butyl dicarbonate Boc_2O, though the reaction seemed rather slow in this case. We also noticed that treatment with $NaBH_4$ prior to *S*-protection was unnecessary if the reaction is conducted under inert atmosphere (entries 3 and 4). The optimal conditions consisted in treating the crude reaction medium with methanol before protection of the thiol moiety with Boc_2O (Table 1, entry 6). We suggest that *S*-desilylation occurs with methanol, the free thiol being further protected. Having this result in hand, we examined whether the method was applicable to aminoacid substrates. Aminoacids in which the carboxyl group was replaced with the heterocycle were obtained according to Scheme 2.

For instance, with glutamic acid, opening of the anhydride with a *N*-cyanomethyl amine such as sarcosine nitrile afforded the amide **4** with a good regioselectivity in favour of the α-carbonyl.[14] To facilitate the purification step, the crude reaction mixture was treated with EDCI/DMAP in anhydrous methanol, leading to the amidoester **4**. Thioacetic acid-mediated addition of H_2S on the nitrile afforded thioamide **5** in a 65% yield. NMR spectra

Table 1 Optimisation study

Entry	Conditions	Yield (%)
1	$NaBH_4$ then $PhCH_2Cl$	R = Bn (**2a**), 24%
2	$NaBH_4$ then PMBCl	R = PMB (**2b**), 32%
3	$NaBH_4$ then Boc_2O, 3 h	R = Boc (**2c**), 31%
4	Boc_2O, 3 h	**2c**, 30%
5	Boc_2O, DMAP, 12 h	**2c**, 50%
6	**MeOH, then Boc_2O, DMAP, 16 h**	**2c, 60%**



Scheme 2 *Reagents and conditions:* i, CH_3–NH–CH_2CN, dioxane; ii, EDCI, DMAP in dry MeOH, 56% overall; iii, 8 equiv CH_3COSH, pyridine, 65%; iv, TMSOTf, Et_3N, CH_2Cl_2, –78 °C to RT; v, MeOH, 15 min; vi, Boc_2O, Et_3N, DMAP, CH_2Cl_2, 53%; vii, 1.5 M HCl, 50% aq dioxane, 40 °C, 100%

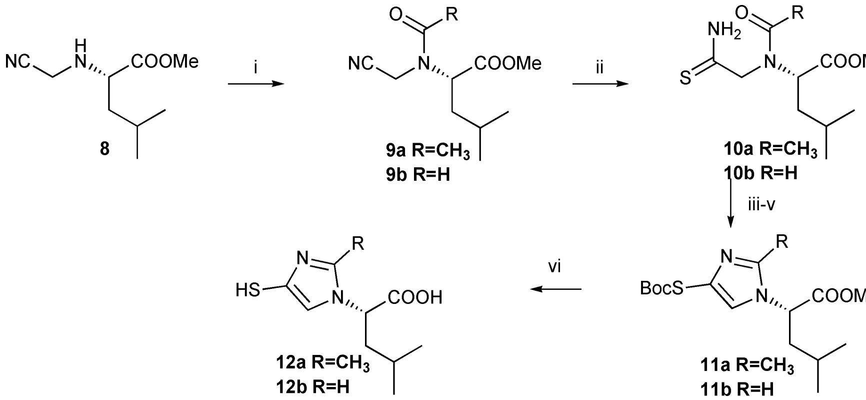
of most cyanomethyl amides and aminothiocarbonyl amides in $CDCl_3$ revealed two rotamers (see experimental section).

It is worth noticing that the use of TMSOTf under our optimized conditions successfully led to compound **6** (53% yield) from a more sensitive functionalized substrate such as **5**. Final deprotection under standard acidic hydrolysis conditions of the thiol, side-chain acid and amine afforded the pure compound **7** as its hydrochloride salt. ^{1}H NMR in D_2O exhibited a rapid exchange of the proton at C5 of the imidazole ring, due to an easy thiol/thione tautomerism.

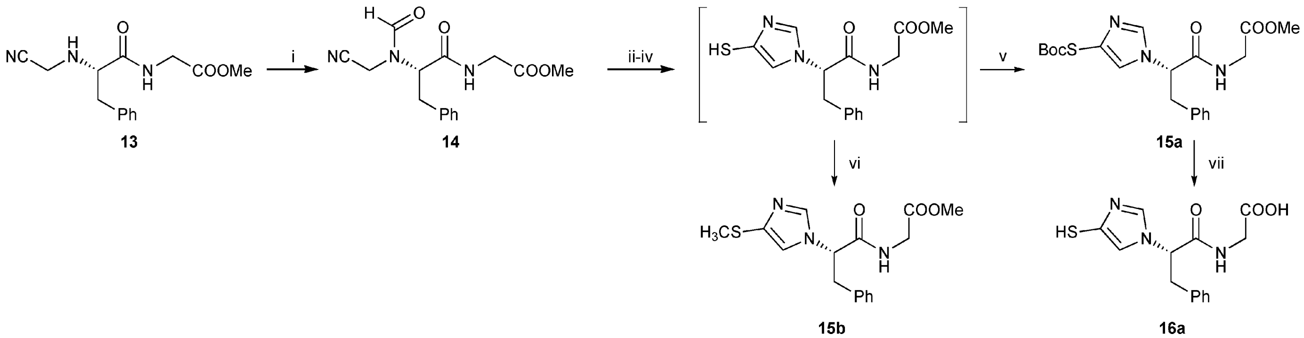
As far as the "*N*-terminus" mimetic was concerned, the cyanomethylamine was prepared directly from the amino group of the aminoacid, as depicted in Scheme 3.

Cyanomethylation of aminoacid methyl esters is a known reaction that can be performed smoothly either by direct alkylation with chloroacetonitrile,[15] or *via* aminomethylation in presence of benzotriazole derivatives.[16] The free cyanomethylamine **8** can be readily purified by flash chromatography on silica gel without noticeable degradation. Subsequent acetylation or formylation was carried out with acetic anhydride or mixed formic acetic anhydride. Compounds **10** were cyclized in good yields with TMSOTf and *S*-Boc protected as described above, to produce the 4-mercaptoimidazole **12** after final acidic hydrolysis. This new isoleucine derivative **12** bears a potential zinc ligand as a surrogate of the amine moiety.

Further applications of this work were devoted to the evaluation of a dipeptidic substrate. We were interested in knowing whether the formation of the imidazole ring was compatible with the presence of the secondary amide bond. Examination of the reactivity of the dipeptide-derived compound **13** proved that our conditions are amenable to starting materials which contain peptidic linkages. In addition, quenching with methyl iodide instead of Boc_2O at the end of the cyclisation process afforded the *S*-methyl derivative **15b**. This allowed an efficient synthesis of both dipeptides **15b** and **16a** (Scheme 4).

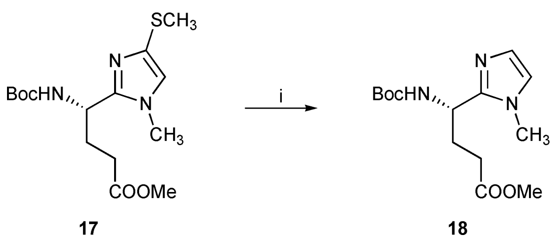


Scheme 3 *Reagents and conditions:* i, RCOOAc, 50% (**9a**), 95% (**9b**); ii, 8 equiv CH_3COSH, pyridine, 78% (**10a**), 71% (**10b**); iii, TMSOTf, Et_3N, CH_2Cl_2, −78 °C to RT; iv, MeOH, 15 min; v, Boc_2O, Et_3N, DMAP, CH_2Cl_2, 60% (**11a**), 66% (**11b**); vi, 1.5 M HCl, 50% aq dioxane, 40 °C, 100%.



Scheme 4 *Reagents and conditions:* i, HCOOH, Ac_2O, 57%; ii, 8 equiv CH_3COSH, pyridine; iii, TMSOTf, Et_3N, CH_2Cl_2, −78 °C to RT; iv, MeOH, 15 min; v, Boc_2O, Et_3N, DMAP, CH_2Cl_2, 42%; vi, CH_3I, Et_3N, 26%; vii, 1.5 M HCl, 50% aq dioxane, 40 °C, 100%.

Recently, 2-substituted 1-benzyl-4-methyl imidazoles were described as acid mimics in aminoacid series. Their preparations used palladium-catalyzed cyclisation of *N*-allyl oxime aminoacid derivatives[17] or electrocyclization of azomethine ylides.[18] We were interested in examining whether our mercaptoimidazoles could serve as precursors of similar, unsubstituted imidazoles. Initial attempts at desulfurisation of *S*-Boc derivatives with Raney nickel were unsuccessful. We could, however, overcome this poor reactivity by using the methylsulfanyl imidazole **17**. The latter was readily converted to the unsubstituted imidazole by treatment with Raney nickel[19] in refluxing ethanol overnight as shown in Scheme 5.



Scheme 5 *Reagents and conditions:* i, Raney nickel, EtOH, reflux, 95%.

Summary and conclusion

This work allowed an efficient and concise synthesis of optically active aminoacid-derived mercaptoimidazoles and imidazoles. These compounds constitute a new series of heterocyclic mimics of aminoacid and peptides, which opens the way towards new enzyme inhibitors or antioxidants. In addition, as the conditions used for the formation of the heterocycle are compatible with peptides, wide screening of compounds with different aminoacid scaffolds is now envisageable.

Experimental

Unless otherwise stated, reactions were performed under a nitrogen atmosphere using freshly distilled solvents. All reactions were monitored by thin-layer chromatography with Merck silica gel 60 F254 pre-coated aluminum plates (0.25 mm). Flash chromatography was performed with indicated solvents using silica gel (particle size 30–63 μm) purchased from Merck. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance at 300 MHz for ^1H and 75 MHz for ^{13}C. Chemical shifts are reported relative to TMS, calibrated with chloroform or deuterium oxide. Coupling constants *J* are given in Hz.

N-(2-Amino-2-thioxoethyl)-*N*-benzylacetamide 1

To a solution of *N*-benzyl-*N*-cyanomethyl acetamide (2.34 g, 12.4 mmol) in CH_2Cl_2 (23 cm^3) was added pyridine (23 cm^3), followed by thioacetic acid (7.57 g, 99.6 mmol). Stirring was kept 16 hr at RT. After concentration *in vacuo*, the residue was purified by flash chromatography through silica gel, using [EtOAc:cyclohexane = 3:2], (R*f* 0.28) as an eluent.

Compound **1** (2.14 g, 77%) was obtained as a stench, pale yellow oil. (Found: C, 57.29; H 8.02; N 12.38. $C_{11}H_{14}N_2OS$ requires C, 57.39; H, 8.21; N, 12.41); ^1H NMR ratio of rotamers 4:1, *major compound* : δ_H(300 MHz; $CDCl_3$) 2.15 (3H, s), 4.29 (2H, s), 4.64 (2H, s), 7.11–7.33 (5H, m), 7.77 (1H, br s), 8.27 (1H, br s); δ_C(75 MHz; $CDCl_3$) 21.9, 53.4, 57.4, 127.0, 128.4, 129.5, 135.8, 173.2, 204.2.

1-Benzyl-2-methyl-4-(Bocsulfanyl)-1*H*-imidazole 2c

General procedure for the preparation of protected mercaptoimidazoles, *e.g.* **2c**:

A solution of *N*-(2-amino-2-thioxoethyl)-*N*-benzylacetamide **1** (489 mg, 2.2 mmol) and Et_3N (1.26 cm^3, 9.0 mmol) in anhydrous CH_2Cl_2 (15 cm^3) was cooled to −78 °C and trimethylsilyl triflate (1.27 cm^3, 6.6 mmol) was added dropwise. After stirring 15 min at −78 °C and 6.5 h at RT, methanol was added and stirring continued during 15 min. The solvents were evaporated and a solution of DMAP (27 mg, 0.22 mmol) and anhydrous Et_3N (2 cm^3, 14.4 mmol) in CH_2Cl_2 (15 cm^3) was added to the residue. To this solution was added dropwise a solution of Boc_2O (1.9 g, 8.8 mmol) in anhydrous CH_2Cl_2 (5 cm^3). After stirring overnight and concentration *in vacuo*, the residue was purified by flash chromatography through silica gel, eluent [EtOAc:cyclohexane:Et_3N= 6:4:0.01], R*f* 0.35, yielding **2c** (400 mg, 60%) as a colourless oil.

(Found: C, 62.93; H, 6.61; N, 9.19; S, 10.69. $C_{16}H_{20}N_2O_2S$ requires C, 63.13; H, 6.62; N, 9.20; S, 10.53); δ_H(300 MHz; $CDCl_3$) 1.40 (9H, s), 2.26 (3H, s), 4.95 (2H, s), 6.99–7.02 (2H, m), 7.22–7.29 (3H, m); δ_C(75 MHz; $CDCl_3$) 13.7, 28.6, 50.5, 85.6, 125.1, 127.1, 127.2, 128.6, 129.5, 135.8, 146.9, 168.3.

1-Benzyl-4-(benzylsulfanyl)-2-methyl-1*H*-imidazole 2a

Compounds **2a** and **2b** were obtained by the same procedure than for **2c**, except that 1.3 molar equiv of the alkylating agent ($PhCH_2Cl$ or 4-methoxybenzyl chloride) was used instead of Boc_2O.

(Found: C, 73.28, H, 6.14, N, 9.25, S, 10.7. $C_{18}H_{18}N_2S$ requires C, 73.43; H, 6.16; N, 9.51; S, 10.89); δ_H(300 MHz; $CDCl_3$) 2.22 (3H, s), 3.90 (2H, s), 4.83 (2H, s), 6.53 (1H, s), 6.86–6.88 (2H, m), 7.08–7.25 (8H, m); δ_C(75 MHz; $CDCl_3$) 13.6, 40.3, 50.1, 124.3, 127.0, 127.1, 128.4, 128.6, 129.36, 129.39, 130.3, 136.2, 138.9, 146.3; [EtOAc:cyclohexane= 3:2, 1% Et_3N]: R*f* 0.38; *m/z* (DCI) 295 (100%), 189 (90%).

1-Benzyl-4-(4-methoxybenzylsulfanyl)-2-methyl-1*H*-imidazole 2b

δ_H(300 MHz; $CDCl_3$) 2.32 (3H, s), 3.76 (3H, s), 3.95 (2H, s), 4.94 (2H, s), 6.64 (1H, s), 6.76 (2H, d, *J* 8.7), 6.96–6.99 (2H, m), 7.11 (2H, d, *J* 8.7), 7.29–7.34 (3H, m); δ_C(75 MHz; $CDCl_3$) 13.6, 39.8, 50.1, 55.6, 114.0, 124.2, 127.0, 128.4, 129.4, 130.5, 130.6, 131.0, 136.3, 146.3, 158.8; *m/z* (DCI) 325 (100%), 121 (30%).

N-Boc-Glutamic anhydride 3

Commercially available *N*-Boc glutamic acid (8 g, 32 mmol) was stirred for 15 min with 80 cm^3 acetic anhydride at 55 °C. Toluene (100 cm^3) was added and the solution was concentrated to dryness. Traces of acetic acid were removed by drying under vacuum over KOH to afford 7.41 g (100%) of anhydride **3**.

δ_H(300 MHz; $CDCl_3$) 1.45 (9H, s), 1.85–2.0 (1H, m), 2.42–2.46 (1H, dd, *J* 12.8, 6.2), 3.01 (1H, dd, *J* 5.5, 2.4), 4.39–4.43 (1H, m), 5.34 (1H, br s); δ_C(75 MHz; $CDCl_3$) 23.6, 28.3, 29.8, 50.9, 81.1, 155.4, 165.3, 167.2; $[\alpha]_D^{20}$ −21 (*c* 1 in CH_2Cl_2).

(*S*)-Methyl-4-(*tert*-butoxycarbonylamino)-5-(*N*-cyanomethyl, *N*-methyl)amino)-5-oxopentanoate 4

A solution of sarcosine nitrile (1.36 g, 19 mmol, obtained from its hydrochloride, by ether extraction from an ice-cold basic solution) in dioxane (5 cm^3) was added dropwise at 10 °C to the anhydride **3** (2.98 g, 13 mmol) in 25 cm^3 dioxane. After 14 h stirring at RT, water (50 cm^3) and ethyl acetate (30 cm^3) were added. The aqueous layer was treated with ice-cold 1M HCl to reach pH 3. The acid was extracted with ethyl acetate and the organic layer dried over $MgSO_4$. After solvent evaporation and drying under vacuum, the residue (2.74 g, 9.1 mmol) was dissolved in absolute methanol (70 cm^3). To this solution were added EDCI (1.92 g, 10.0 mmol) and DMAP (110 mg, 0.91 mmol). Stirring was kept during 5 h at RT. After solvent evaporation, extractive workup with ethyl acetate/satd NH_4Cl, and drying over $MgSO_4$, the ester **4** (2.3 g, 56%) was obtained as a pale brown oil.

δ_H(300 MHz; $CDCl_3$), major rotamer: 1.39 (9H, s), 2.31–2.50 (4H, m), 3.30 (3H, s), 3.71 (3H, s), 4.20–4.41 (3H, m), 5.26–5.34 (1H, m); δ_C(75 MHz; $CDCl_3$) 27.7, 27.9, 28.3, 35.2, 35.3, 35.7, 52.5, 80.1, 115.4, 155.6, 172.3, 172.8.

(*S*)-Methyl 5-(*N*-(2-amino-2-thioxoethyl), *N*-(methyl)amino)-4-(*tert*-butoxycarbonylamino)-5-oxopentanoate 5

Starting from compound **4** (2.0 g, 6.38 mmol), the thioamide **5** (1.44 g, 65%) was obtained following the procedure described for **1**.

(Found: C, 48.32, H, 7.39, N, 12.35, S, 9.16. $C_{14}H_{25}N_3O_5S$ requires C, 48.40; H, 7.25; N, 12.09; S, 9.23); δ_H(300 MHz; $CDCl_3$): 1.38 (9H, s), 1.57–1.65 (1H, m), 2.26–2.61 (3H, m), 2.98 (3H, s), 3.78 (3H, s), 3.96 (1H, d, *J* 17.1), 4.38–4.45 (1H, m), 5.03 (1H, d, J 17.0), 5.43 (1H, d, *J* 8.1), 7.98 (1H, br s), 8.63 (1H, br s); δ_C(75 MHz; $CDCl_3$) 28.2, 28.3, 29.0, 36.0, 52.1, 52.7, 59.0, 80.4, 156.1, 172.4, 173.1, 203.5; $[\alpha]_D^{20}$ +20.61 (*c* 0.97 in CH_2Cl_2); *m/z* (DCI) 348 (100%).

(*S*)-Methyl 4-(*tert*-butoxycarbonylamino)-4-(4-(*tert*-butoxy carbonylthio)-1-methyl-1*H*-imidazol-2-yl)butanoate 6

Following the procedure given for **2c** and starting from **5** (1.21 g, 3.48 mmol), **6** (790 mg, 53%) was obtained as a pale yellow oil. (Found: C, 53.08; H, 7.39; N, 9.86; S, 7.89. $C_{19}H_{31}N_3O_6S$ requires C, 53.13; H, 7.27; N, 9.78; S, 7.47); δ_H(300 MHz; $CDCl_3$): 1.27 (9H, s), 1.42 (9H, s), 2.11–2.21 (1H, m), 2.30–2.42 (1H, m), 2.72 (2H, d, *J* 8.0), 3.55 (3H, s), 3.69 (3H, s), 4.30–4.37 (1H, m), 5.34 (1H, d, *J* 7.6), 7.06 (1H, s); δ_C(75 MHz; $CDCl_3$) 28.3, 28.4, 29.8, 30.2, 32.9, 52.6, 53.2, 80.0, 85.4, 124.6, 127.6, 148.6, 155.6, 168.2, 172.7; $[\alpha]_D^{20}$ +9.41 (*c* 1.2 in CH_2Cl_2).

(*S*)-4-Amino-4-(4-mercapto-1-methyl-1*H*-imidazol-2-yl)butanoic acid hydrochloride 7

Protected compound **6** (95 mg, 0.22 mmol) was dissolved in dioxane under argon. A degased solution of 3M hydrochloric acid was added and the reaction mixture stirred 13 h at 40 °C. Evaporation to dryness followed by freeze-drying from water afforded 56 mg (100%) of the free thiol **7**.

δ_H(300 MHz; D_2O) 2.32–2.39 (2H, m), 3.06–3.24 (2H, m), 3.75 (3H, s), 4.11 (1H, t, *J* 6.0), 7.51 (1H, br s); δ_C(75 MHz; D_2O) 21.4, 26.8, 34.8, 52.8, 123.3, 149.1, 172.1; $[\alpha]_D^{20}$ +24.3 (*c* 1 in abs EtOH).

(*S*)-Methyl 2-(cyanomethylamino)-4-methylpentanoate 8

A 1 M aqueous solution of NaOH (27.6 cm^3) was added dropwise to a suspension of valine methyl ester (5 g, 27.6 mmol) and benzotriazol (3.61 g, 30.3 mmol) in methanol (50 cm^3). Formaline (2.1 cm3, 5.5 mmol) was added and the reaction medium was stirred 4 hr at RT. Extraction with petroleum ether followed by drying over $MgSO_4$, filtration and evaporation gave an oil (6.87 g) which was redissolved in DMSO (50 cm^3). Sodium cyanide (1.64 g, 33.6 mmol) was added. After stirring 40 hr at RT, ethyl acetate was added, the organic layer was decanted off and washed with Na_2CO_3, brine and dried over $MgSO_4$. The aqueous residues were treated with bleach before discarding.

(Found: C, 58.36; H 8.81; N 15.94. $C_9H_{16}N_2O_2$ requires C, 58.67; H, 8.75; N, 15.31); δ_H(300 MHz; $CDCl_3$) 0.92 (6H, d, *J* 6.6), 1.41–1.58 (2H, m), 1.67–1.82 (2H, m), 3.39 (1H, dd, *J* 8.1, 6.2), 3.54 (1H, d, *J* 17.4), 3.64 (1H, d, *J* 17.4), 3.75 (3H, s); δ_C(75 MHz; $CDCl_3$) 21.8, 22.7, 24.6, 35.9, 42.1, 52.0, 58.7, 117.5, 174.8; $[\alpha]_D$= −32.0 (*c* 1, CH_2Cl_2).

(*S*)-Methyl 2-(*N*-(cyanomethyl)acetamido)-4-methyl pentanoate 9a

Aminonitrile **8** (700 mg, 3.78 mmol) in CH_2Cl_2 (5 cm^3) was treated with 640 mg (6.22 mmol) of acetic anhydride. After 2 h stirring at RT, evaporation and flash chromatography through silica gel, eluent [EtOAc:cyclohexane = 6:4], R*f* 0.55 gave compound **9a** (425 mg, 50%).

(Found: C, 58.43; H 8.29; N 12.55. $C_{11}H_{18}N_2O_3$ requires C, 58.39; H, 8.02; N, 12.38); δ_H(300 MHz; $CDCl_3$): two rotamers, ratio 3:2 in this solvent: *major:* 0.95–1.02 (6H, m), 1.64–1.89 (3H, m), 2.30 (3H, s), 3.72 (3H, s), 4.09 (1H, d, *J* 15.0), 4.36 (1H, d, *J* 15.0), 4.45 (1H, dd, *J* 9.1, 6.0); *minor:* 0.95–1.02 (6H, m), 1.64–1.89 (3H, m), 2.20 (3H, s), 3.77 (3H, s), 4.28 (2H, s), 5.41 (1H, dd, *J* 9.9, 5.4); δ_C(75 MHz; $CDCl_3$) 24.7, 30.4, 38.2, 52.6, 54.0, 115.7, 171.0, 171.1; $[\alpha]_D^{20}$ −28.4 (*c* 1.09 in CH_2Cl_2).

(*S*)-Methyl 2-(*N*-(cyanomethyl)formamido)-4-methyl pentanoate 9b

An equimolar mixture of formic acid (9.7 g, 212 mmol) and acetic anhydride was heated at 65 °C during 10 minutes. After cooling to 0 °C, this solution was added to aminonitrile **8** (1.5 g, 8.14 mmol) in CH_2Cl_2 (5 cm^3). After 10 min stirring at RT, treatment with ice-cold water (240 cm^3) and extractive work up with CH_2Cl_2 followed by washing with satd $NaHCO_3$ gave compound **9b** (1.6 g, 95%).

(Found: C, 56.51; H, 7.55; N, 13.69. $C_{10}H_{16}N_2O_3$ requires C, 56.59; H, 7.60; N, 13.20); δ_H(300 MHz; $CDCl_3$) two rotamers, ratio 4:1 in this solvent: *major:* 0.95–1.02 (6H, m), 1.59–1.69 (1H, m), 1.76–1.87 (2H, s), 3.78 (3H, s), 4.19–4.39 (3H, m), 8.15 (1H, s); δ_C(75 MHz; $CDCl_3$) 21.0, 22.7, 29.2, 37.9, 52.8, 58.2, 114.9, 162.8, 170.9; $[\alpha]_D^{20}$ −24.4 (*c* 0.53 in CH_2Cl_2).

(*S*)-Methyl 2-(*N*-(2-amino-2-thioxoethyl)acetamido)-4-methylpentanoate 10a

Following the procedure described for the formation of **1**, compound **9a** (250 mg, 1.10 mmol) was treated with thioacetic acid, leading to **10a** (223 mg, 78%).

(Found: C, 45.66; H, 7.21; N, 8.71; S, 8.20. $C_{11}H_{20}N_2O_3S$ requires C, 50.75; H, 7.74; N, 10.76; S, 12.32); δ_H(300 MHz; $CDCl_3$): two rotamers, ratio 6.7:1 in this solvent: *major:* 0.93–0.99 (6H, m), 1.41–1.58 (1H, m), 1.80 (2H, t, *J* 7.4), 2.08 (3H, s), 3.8 (3H, s), 4.27 (1H, t, *J* 7.0), 4.36 (1H, d, *J* 19.4), 4.52 (1H, d, *J* 19.4), 7.83 (1H, br s), 10.1 (1H, br s); δ_C(75 MHz; $CDCl_3$) 22.0, 22.1, 23.2, 25.2, 38.0, 53.3, 59.4, 172.2, 175.0, 203.5; $[\alpha]_D^{20}$ −63.8 (*c* 0.8 in CH_2Cl_2).

(*S*)-Methyl 2-(*N*-(2-amino-2-thioxoethyl)formamido)-4-methylpentanoate 10b

Following the procedure described for the formation of **1**, compound **9b** (1.6 g, 7.54 mmol) was treated with thioacetic acid, leading to **10b** (1.31 g, 71%).

(Found: C, 48.68; H, 7.39; N, 11.33; S, 12.67. $C_{11}H_{14}N_2OS$ requires C, 48.76; H, 7.37; N, 11.37; S, 13.02; δ_H(300 MHz; $CDCl_3$): two rotamers, ratio 1.25:1 in this solvent: *major:* 0.82–0.92 (6H, m), 1.55–1.65 (3H, m), 3.60 (3H, s), 3.98–4.34 (2H, m), 4.78 (1H, m), 8.14 (1H, s), 9.37 (1H, br s), 9.80 (1H, br s); *minor:* 0.82–0.92 (6H, m), 1.55–1.65 (3H, m), 3.65 (3H, s), 3.98–4.34 (2H, m), 4.43 (1H, m), 8.24 (1H, s), 8.91 (1H, br s), 9.70 (1H, br s).

(*S*)-Methyl 2-(4-(*tert*-butoxycarbonylthio)-2-methyl-1*H*-imidazol-1-yl)-4-methylpentanoate 11a

Following the procedure described for the formation of **2**, compound **10a** (160 mg, 0.62 mmol) led to protected mercaptoimidazole **11a** (127 mg, 60%).

(Found: C, 55.49; H, 7.78; N, 7.76; S, 9.25. $C_{16}H_{26}N_2O_4S$ requires C, 56.12; H, 7.65; N, 8.18; S, 9.36); δ_H(300 MHz; $CDCl_3$): 0.92 (6H, m), 1.47 (9H, s), 1.47 (1H, m), 1.84–2.03 (2H, m), 2.39 (3H, s), 3.73 (3H, m), 4.67 (1H, dd, *J* 9.0, 6.6), 7.23 (1H, s); δ_C(75 MHz; $CDCl_3$) 13.6, 21.8, 22.7, 24.6, 28.3, 41.2, 53.1, 56.9, 85.3, 123.7, 125.6, 146.3, 167.6, 170.2; $[\alpha]_D^{20}$ −3.27 (*c* 0.61 in CH_2Cl_2); *m*/*z* (DCI) 343 (100%), 299 (10%), 287 (25%).

(*S*)-Methyl 2-(4-(*tert*-butoxycarbonylthio)-1*H*-imidazol-1-yl)-4-methylpentanoate 11b

Following the procedure described for the formation of **2**, compound **10b** (299 mg, 1.21 mmol) led to protected mercaptoimidazole **11b** (262 mg, 66%).

(Found: C, 55.04; H, 7.51; N, 8.57; S, 9.56. $C_{15}H_{24}N_2O_4S$ requires C, 54.86; H, 7.37; N, 8.53; S, 9.76); δ_H(300 MHz; $CDCl_3$): 0.90–0.94 (6H, m), 1.38–1.47 (1H, m), 1.47 (9H, s), 1.91–1.97 (2H, m), 3.74 (3H, m), 4.74 (1H, dd, *J* 8.7, 7.2), 7.29 (1H, d, *J* 1.3), 7.62 (1H, d, *J* 1.3); δ_C(75 MHz; $CDCl_3$) 21.6, 22.6, 24.6, 28.2, 41.7, 53.1, 60.5, 85.4, 124.6, 127.6, 137.8, 167.3, 170.1; *m*/*z* (DCI) 329 (100%), 273 (90%), 229 (20%).

(*S*)-2-(4-Mercapto-2-methyl-1*H*-imidazol-1-yl)-4-methyl pentanoic acid 12a

Hydrolysis was carried out by stirring a solution of compound **11b** (86 mg, 0.26 mmol) in 1.5 M HCl in H_2O:dioxane 1:1 at 40 °C overnight. After concentration *in vacuo*, the residue was taken up in water and freeze-dried. 56 mg (100%) of compound **12b** were obtained as a pale yellow foam.

δ_H(300 MHz; DMSO) 0.85 (3H, d, *J* 6.6), 0.88 (3H, d, *J* 6.6), 1.17–1.20 (1H, m), 1.81–2.02 (2H, m), 2.40 (3H, s), 4.62 (1H, dd, *J* 9.8, 6.0), 7.20 (1H, s); δ_C(75 MHz; DMSO) 11.6, 21.3, 22.6, 24.3, 58.0, 126.2, 148.3, 170.0.

(*S*)-2-(4-Mercapto-1*H*-imidazol-1-yl)-4-methyl pentanoic acid 12b

Hydrolysis was carried out by stirring a solution of compound **11b** (86 mg, 0.26 mmol) in 1.5 M HCl in H_2O:dioxane 1:1 at 40 °C overnight. After concentration *in vacuo*, the residue was taken up in water and freeze-dried. 56 mg (100%) of compound **12b** were obtained as a pale yellow foam.

δ_H(300 MHz; D_2O) 0.76 (3H, d, *J* 6.6), 0.78 (3H, d, *J* 6.6), 1.38–1.47 (1H, m), 1.88–2.05 (2H, m), 5.15 (1H, dd, *J* 10.4, 5.5), 7.51 (1H, d, *J* 1.3), 8.79 (1H, s); δ_C(75 MHz; D_2O) 20.5, 22.2, 24.7, 39.0, 62.1, 125.5, 127.7, 138.8, 141.4, 147.5, 172.9. $[\alpha]_D^{20}$ +72.6 (*c* 1.22 in EtOH).

(*S*)-Methyl 2-(2-(cyanomethylamino)-3-phenyl propanamido) acetate 13

The dipeptide Phe-Gly-OMe was treated as described for **8**.

(Found: C, 60.57; H, 6.17; N, 15.86. $C_{14}H_{17}N_3O_3$ requires C, 61.08; H, 6.22; N, 15.26); δ_H(300 MHz; DMSO + TFA) 3.11 (2H, d, *J* 6.6), 3.63 (3H, s), 3.92 (2H, d, *J* 5.6), 4.17 (2H, s), 7.24–7.31 (5H, m), 7.40 (1H, dd, *J* 6.4, 3.0), 7.87 (1H, dd, *J* 6.2, 3.0), 9.11 (1H, t, *J* 5.6); δ_C(75 MHz; DMSO + TFA) 36.2, 41.0, 52.1, 60.7, 125.6, 128.8, 129.8, 134.3, 167.4, 169.9; $[\alpha]_D^{20}$ −28.9 (*c* 0.94 in EtOH).

(*S*)-Methyl 2-(2-(*N*-(cyanomethyl)formamido)-3-phenyl propanamido)acetate 14

Aminonitrile **13** was treated with HCOOH/Ac_2O, using the same protocol than for **9b**.

(Found: C, 59.62; H 5.14; N 13.68. $C_{15}H_{17}N_3O_4$ requires C, 59.40; H, 5.65; N, 13.85); δ_H(300 MHz; DMSO + TFA) 3.01 (1H, dd, *J* 14.3, 10.1), 3.21 (1H, dd, *J* 14.3, 5.5), 3.61 (3H, s), 3.89 (1H, d, *J* 5.9), 4.19 (1H, d, *J* 17.5), 4.34 (1H, d, *J* 17.5), 4.64–4.69 (1H, m), 7.18–7.27 (5H, m), 7.92 (1H, s), 8.79 (1H, t, *J* 5.7); ^{13}C NMR ($CDCl_3$, 75 MHz) 29.8, 36.2, 41.4, 52.7, 62.6, 115.3, 127.7, 129.1, 129.3, 135.5, 168.6, 168.9, 170.0; $[\alpha]_D^{20}$ −62.6 (*c* 1 in CH_2Cl_2).

(*S*)-Methyl 2-(2-(*N*-(2-amino-2-thioxoethyl)formamido)-3-phenylpropanamido)acetate

Obtained by treatment of the aminonitrile **14** with thioacetic acid (see procedure for **1**).

(Found: C, 53.42; H 5.82; N 12.32, S 9.43. $C_{15}H_{19}N_3O_4S$ requires C, 53.40; H, 5.68; N, 12.45; S, 9.50); δ_H(300 MHz; DMSO + TFA) two rotamers, ratio 3:1, *major:* 3.05–3.26 (2H, m), 3.60 (3H, s), 3.84 (2H, d, *J* 5.7), 4.00 (1H, d, *J* 17.5), 4.09 (1H, d, *J* 17.5), 4.54 (1H, t, *J* 7.7), 7.19–7.30 (5H, m), 8.07 (1H, s), 8.77 (1H, t, *J* 5.6), 8.87 (1H, br s), 9.70 (1H, br s); δ_C(75 MHz; $CDCl_3$) 37.3, 41.9, 52.7, 54.7; 63.6, 127.9, 129.6, 130.1, 137.7, 164.8, 170.8, 171.3, 204.2; $[\alpha]_D^{20}$ −95.1 (*c* 1 in CH_2Cl_2); *m/z* (DCI) 338 (100%), 304, 265, 90, 76.

(*S*)-Methyl 2-(2-(4-(*tert*-butoxycarbonylthio)-1*H*-imidazol-1-yl)-3-phenylpropanamido)acetate 15a

Thioamide resulting from treatment of **14** (250 mg, 0.74 mmol) was dehydrated to give the *S*-Boc mercaptoimidazole **15a** (131mg, 42%), using the procedure described for **2c**.

(Found: C, 57.69; H, 5.76; N, 9.89; S, 7.75. $C_{20}H_{25}N_3O_5S$ requires C, 57.26; H, 6.01; N, 10.02; S, 7.64); δ_H(300 MHz; $CDCl_3$), two rotamers, ratio 9:1, *major:* 1.48 (9H,s), 3.17 (1H, dd, *J* 14.0, 9.4), 3.50 (1H, dd, *J* 14.0, 5.7), 3.71 (3H, s), 3.99 (2H, t, *J* 5.5), 4.81 (1H, dd, *J* 9.2, 5.8), 6.99–7.02 (2H, m), 7.16–7.22 (3H, m), 7.29 (1H, s), 7.32 (1H, d, *J* 1.1); δ_C(75 MHz; $CDCl_3$) 28.3, 39.3, 41.5, 52.5, 62.9, 85.8, 124.7, 127.4, 127.7, 128.9, 129.0, 135.8, 138.5, 154.8, 168.4, 169.7; $[\alpha]_D^{20}$ −55.3 (*c* 0.85 in CH_2Cl_2); *m/z* (DCI) 420, 376, 320, 222, 79.

(*S*)-Methyl 2-(2-(4-(methylthio)-1*H*-imidazol-1-yl)-3-phenyl propanamido)acetate 15b

The procedure for the formation of **2c** was used, except that methyl iodide (2 molar equiv) was used instead of Boc_2O.

δ_H(300 MHz; $CDCl_3$): 2.35 (3H, s), 3.17 (1H, dd, *J* 14.1, 9.6), 3.51 (1H, dd, *J* 14.1, 5.7), 3.70 (3H, s), 4.00 (2H, d, *J* 5.4), 4.90 (1H, dd, *J* 9.4, 5.5), 6.97–7.00 (2H, m), 7.06 (1H, s), 7.19–7.21 (3H, m), 7.26 (1H, s), 7.88 (1H, t, *J* 5.4); δ_C(75 MHz; $CDCl_3$) 18.1, 39.1, 41.3, 52.4, 62.5, 118.3, 127.3, 128.7, 128.8, 135.9, 136.1, 137.6, 168.9, 169.9; *m/z* (ESI) 334; $[\alpha]_D^{20}$ −54.5 (*c* 0.22 in CH_2Cl_2).

(*S*)-2-(2-(4-Mercapto-1*H*-imidazol-1-yl)-3-phenyl propanamido)acetic acid 16a

The procedure used for compound **7** was applied.

δ_H(300 MHz; D_2O) 3.32 (1H, dd, *J* 13.9, 10.0), 3.54 (1H, dd, *J* 13.9, 5.9), 3.93 (1H, d, *J* 17.9), 4.02 (1H, d, *J* 17.9), 5.38 (1H, dd, *J* 9.0, 6.0), 7.14–7.17 (2H, m), 7.29–7.31 (3H, m), 7.44 (1H, s), 8.65 (1H, s); δ_C(75 MHz; $CDCl_3$) 38.4, 41.5, 63.7, 127.9, 128.0, 129.0, 129.2, 129.3, 134.9, 138.5, 169.6, 173.0; $[\alpha]_D^{20}$ −96.9 (*c* 0.8 in CH_2Cl_2); *m/z* (ESI) 304.

(*S*)-Methyl 4-(*tert*-butoxycarbonylamino)-4-(1-methyl-4-(methylthio)-1*H*-imidazol-2-yl)butanoate 17

Following the procedure described for the formation of **15b**, thioamide **5** (444 mg, 1.28 mmol) led to protected mercaptoimidazole **17** (198 mg, 45%).

δ_H(300 MHz; $CDCl_3$): 1.43 (9H, s), 2.16–2.38 (2H, m), 2.39 (3H, s), 2.71 (2H, t, *J* 7.4), 3.51 (3H, s), 3.72 (3H, s), 4.35 (1H, m), 5.77 (1H, br d, *J* 7.0), 6.77 (1H, s); δ_C(75 MHz; $CDCl_3$) 19.0, 23.3, 28.7, 29.9, 32.9, 52.7, 53.9, 80.1, 121.9, 133.2, 148.3, 156.0, 173.1.

(*S*)-Methyl 4-(*tert*-butoxycarbonylamino)-4-(1-methyl-1*H*-imidazol-2-yl)butanoate 18

Refluxing sulfide **17** (99 mg, 0.29 mmol) in ethanol (15 cm^3), in the presence of 50 mg of Raney nickel (50% in water) during

14 h, followed by filtration through celite, gave 82 mg (95%) of compound **18**.

δ_H(300 MHz; $CDCl_3$): 1.43 (9H, s), 2.11–2.37 (2H, m), 2.72 (2H, t, *J* 7.7), 3.54 (3H, s), 3.70 (3H, s), 4.35 (1H, m), 5.60 (1H, br d, *J* 7.7), 6.77 (1H, s), 6.89 (1H, s); δ_C(75 MHz; $CDCl_3$) 22.8, 28.4, 30.1, 32.6, 52.5, 53.3, 80.2, 120.8, 127.1, 147.0, 155.7, 172.9.

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References

1 S. Hanessian, G. McNaughton-Smith, H.-G. Lombart and W. D. Lubell, *Tetrahedron*, 1997, **53**, 12789; J. Gante, *Angew. Chem. Int. Ed. Engl.*, 1994, **33**, 1699.
2 R. E. Melendez and W. D. Lubell, *J. Am. Chem. Soc.*, 2004, **126**, 6759.
3 S. Couve-Bonnaire, D. Cahard and X. Pannecoucke, *Org. Biomol. Chem.*, 2007, **5**, 1151.
4 M. H. V. Ramana Rao, E. Pinyol and W. D. Lubell, *J. Org. Chem.*, 2007, **72**, 736 and references cited therein.
5 B. P. Roques, M.-C. Fournie-Zaluski, E. Soroca, J.-M. Lecomte, B. Malfroy, C. Llorens and J.-C. Schwartz, *Nature*, 1980, **288**, 286; B. P. Roques, F. Noble, V. Dauge, M.-C. Fournie-Zaluski and A. Beaumont, *Pharmacol. Rev.*, 1993, **45**, 87; C. David, L. Bischoff, H. Meudal, A. Mothe, N. De Mota, S. DaNascimento, C. Llorens-Cortes, M.-C. Fournie-Zaluski and B. P. Roques, *J. Med. Chem.*, 1999, **42**, 5197.
6 B. W. Matthews, *Acc. Chem. Res.*, 1988, **21**, 333.
7 T. P. Holler, F. Ruan, A. Spaltenstein and P. B. Hopkins, *J. Org. Chem.*, 1989, **54**, 4570; C. E. Hand and J. F. Honek, *J. Nat. Prod.*, 2005, **68**, 293.
8 P. Deprez, E. Mandine, A. Vermond and D. Lesuisse, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 1287.
9 A. Spaltenstein, T. P. Holler and P. B. Hopkins, *J. Org. Chem.*, 1987, **52**, 2977.
10 V. Zoete, F. Bailly, J.-P. Catteau and J.-L. Bernier, *J. Chem. Soc. Perkin Trans.*, 1997, **1**, 2983.
11 F. Bailly, N. Azaroual and J.-L. Bernier, *Bioorg. Med. Chem.*, 2003, **11**, 4623.
12 K. Ikeda, S. Kitani, K. Sato, T. Suzuki, C. Hosokawa, Y. Suzuki, K. Tanaka and M. Sato, The protocol described in the following article, *Carbohydrate Res.*, 2004, **339**, 1367 claims 1 molar equiv of AcSH, whereas the amount used therein corresponds to 8 molar equiv.
13 T. P. Holler and P. B. Hopkins, *J. Am. Chem. Soc.*, 1988, **110**, 4837.
14 X. Huang, X. Luo, Y. Roupioz and J. Keillor, *J. Org. Chem.*, 1997, **62**, 8821.
15 H. Tokuyama, T. Kuboyama, A. Amano, T. Yamashita and T. Fukuyama, *Synthesis*, 2000, **9**, 1299.
16 A. R. Katritzky, N. Kirichenko, B. V. Rogovoy and H.-Y. He, *J. Org. Chem.*, 2003, **68**, 9088.
17 S. Zaman, K. Mitsuru and A. D. Abell, *Org. Lett.*, 2005, **7**, 609.
18 O. A. Attanasi, P. Davoli, G. Favi, P. Filippone, A. Forni, G. Moscatelli and F. Prati, *Org. Lett.*, 2007, **9**, 3461.
19 Y. Tominaga, Y. Shiroshita, M. Kawabe, H. Goto, Y. Oniyama and Y. Matsuda, *Heterocycles*, 1985, **23**, 2531.