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A new method for peptide synthesis in the N→C direction: amide assembly through silver-promoted reaction of thioamides†

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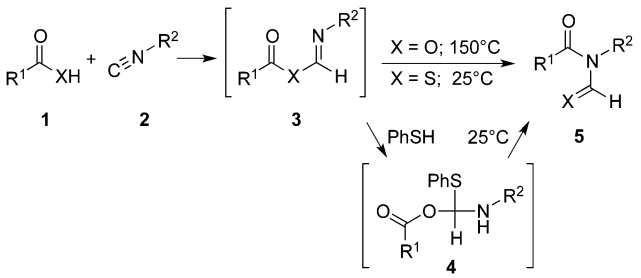
The Ag(I)-promoted coupling of amino acids and peptides with amino ester thioamides generates peptide imides without epimerisation. The peptide imides undergo regioselective hydrolysis under mild conditions to generate native peptides. This method was employed to prepare the pentapeptide thymopentin in the N→C direction, in high yield and purity.

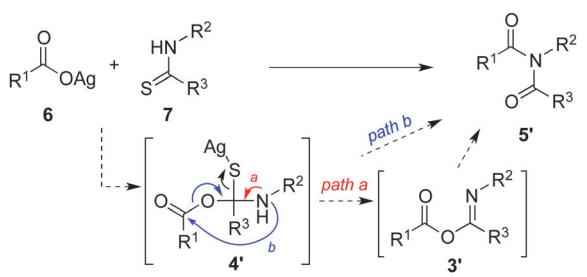
The quest for rapid and direct synthesis of peptides and proteins under mild conditions has led to a vast number of modern methods for the construction of amide bonds.[1] The search for chemoselectivity has led to the development of methods that incorporate non-traditional coupling partners such as alcohols,[2] bromonitroalkanes,[3] ketoacids,[4] isonitriles[5] and others.[6] Further, the development methods amenable to the N→C direction synthesis of peptides is of interest for the direct preparation of C-terminally modified peptides.[7] Standard coupling methods involving generation of an active ester at the peptide C-terminus suffer from epimerisation of the C-terminal residue.[7]

Danishefsky has developed a peptide coupling strategy through the reaction of isonitriles with thioacids (Scheme 1, X = S).[5] In this process, the thioacid and isonitrile react to generate a thioformimidate carboxylate mixed anhydride intermediate **3** (thioFCMA, X = S), which can undergo trapping with an exogenous amine to generate an amide, or a 1,3-acyl transfer to generate a thioformimide **5**.[1d,5] The closely related coupling of isonitriles with carboxylic acids (Scheme 1, X = O), only undergoes the 1,3-acyl transfer at temperatures above 150 °C to generate formimides **5**.[8] Interestingly, it was found that addition of thiophenol results in generation of the imide product **5** at room temperature, albeit in low yield (10–25%).[9] A tetrahedral intermediate **4** was invoked to rationalise the rearrangement occurring at room temperature.



Scheme 1 Imides from isonitriles and carboxylic acids or thioacids.

In the quest for new methods of amide formation utilising novel coupling partners, we hypothesised that the reaction of a thioamide with a carboxylate in the presence of a thiophilic metal such as Ag(I) would generate a tetrahedral species similar to **4**, which would provide access to imides **5**. This approach would avoid the concurrent formation of thioacetal products, a major limitation in the isonitrile route.[9] Indeed, the reaction of secondary thioamides with silver carboxylates has been shown to generate imides.[10] The mechanism of this transformation is proposed to proceed through both a tetrahedral intermediate **4′** and a mixed anhydride intermediate **3′**, closely related to those observed in the isonitrile route, with final 1,3-acyl transfer generating the imide **5** (path a, Scheme 2).[10] Despite the analogous reaction pathways, the coupling of thioamides and carboxylic acids to generate peptides has not been exploited.



Scheme 2 Proposed mechanism of imide formation from thioamides.[10]

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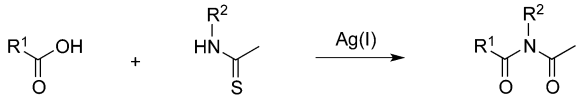
Table 1 Optimisation of imide formation[a]



8 R^1 = Me **9** R^2 = Bn **10**
11G R^1 = $PhthNCH_2$ **12G** R^2 = CH_2CO_2Me

Entry	Acid	Thioamide	Ag(I)	Stoichiometry Acid : thioamide : Ag(I)	Yield (%)
1	—	**9**	AgOAc	—:1:2	89
2	**8**	**9**	Ag_2CO_3	1:1:2	85
3	**8**	**9**	Ag_2CO_3	1:1:1	40
4	**8**	**9**	Ag_2SO_4	1:1:2	0 (25[b])
5	**11G**	**9**	Ag_2CO_3	1:1:2	66 (61[c])
6	**11G**	**9**	Ag_2CO_3	1:1:2	60[d]
7	**11G**	**9**	Ag_2CO_3	2:1:2	32
8	**11G**	**9**	Ag_2CO_3	1:1:4	56
9	**11G**	**9**	Ag_2CO_3	1:2:4	69
10	**11G**	**12G**	Ag_2CO_3	1:1:2	66
11	**11G**	**12G**	HgO	1:1:1.5[e]	68
12	**11G**	**12G**	$CuCO_3$	1:1:1.5[f]	0
13	**11G**	**12G**	Ag_2CO_3	1:1.5:3	76 (82[g])
14	**11G**	**12G**	Ag_2CO_3	1:2:4	78

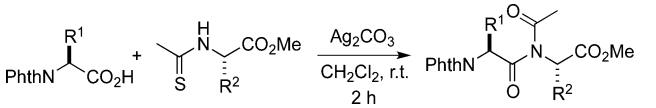
[a] Conditions: acid, thioamide, Ag(I) salt in CH_2Cl_2, RT, 2 h. [b] With 3 equiv. Et_3N. [c] With 10 equiv. H_2O. [d] Solvent; CH_3CN. [e] 1.5 equiv. HgO. [f] 1.5 equiv. $CuCO_3$. [g] Solvent; DMF.

We herein describe the coupling of thioamides and carboxylic acids to generate imides and ultimately amide products, including application to the preparation of peptides. This silver-promoted reaction proceeds rapidly at room temperature, without epimerisation. We show that selective imide hydrolysis is feasible to furnish amide products. The application of this process to the N→C direction synthesis of peptides is highlighted through preparation of the pentapeptide thymopentin.

Synthesis of the thioamide coupling partners was achieved in good to excellent yields (70–92%) through several methods: by acylation of the corresponding amine followed by treatment Lawesson's reagent, or alternatively by direct thioacetylation of the amine with Rapoport's reagent[11] or ethyldithioacetate (see ESI†).

A model coupling of *N*-benzylthioacetamide **9** with silver acetate generated the imide **10** (R^1 = Me, R^2 = Bn) in excellent yield (Table 1, entry 1). The combination of acetic acid and silver carbonate gave similar results to the use of silver acetate (entry 2), demonstrating that pre-formed silver carboxylates are not necessary for this process. Non-basic Ag(I) salts such as Ag_2SO_4 were only effective if base was added (entry 4). Hg(II) was similarly effective as Ag(I), while Cu(II) was less effective (entries 11 and 12).[10] Use of *N*-phthaloyl glycine **11G** as the acid component gave the glycine imide **10** (R^1 = $PhthCH_2$, R^2 = Bn) in reasonable yield (entry 5). Optimization of this reaction was undertaken with regard to solvent and stoichiometry. CH_2Cl_2, acetonitrile and DMF were all suitable solvents for the reaction (entries 5 and 13). The reaction was tolerant of water, with addition of 10 equiv. of water having no noticeable effect on the yield. At least one equivalent of Ag_2CO_3 (2 equiv. Ag^I) was required for good yields, while excess carboxylic acid was detrimental (entries 2 and 3; 6 and 7). An excess of both thioamide and Ag_2CO_3 gave optimum yield (entry 9). Employing the glycine thioacetamide **12G**, as little as 1.5 equiv. thioamide and Ag_2CO_3 furnished the imide in optimum yield (entries 13 and 14). While these reactions are typically complete in 10–15 minutes at room temperature, they were routinely left for two hours.

Table 2 Synthesis of dipeptide imides[a]



11 **12** **10**

11 R^1	**12** R^2 H (G)	Me (A)	Bn (F)	iPr (V)	iBu (L)
H (G)	76	99	94	99	95
Me (A)	84	95	83	95	99
Bn (F)	72	86	79	99	81
iPr (V)	76	71	71	94	99
iBu (L)	70	92	75	90	84

[a] Isolated yields (%). Conditions: 1.0 equiv. **11**, 1.5 equiv. **12**, 1.5 equiv. Ag_2CO_3.

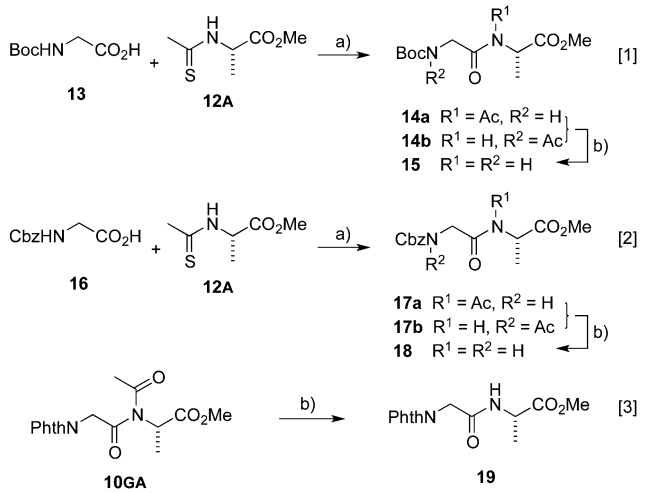
The synthesis of protected dipeptides from *N*-phthaloyl amino acids **11** and thioacetylated amino esters **12** was investigated under the optimised conditions (Table 2). In all cases the yields obtained for the imide products were good to excellent (71–100%), with the only byproducts being silver sulfide and the amide precursor. Notably, excellent yields were obtained even when sterically hindered amino acids such as valine and leucine were employed.

The dipeptide imides **10** were isolated as single stereoisomers, with no evidence of epimerisation. Chiral HPLC analysis of dipeptide imide **10AG** confirmed the absence of epimerisation during this coupling, with $<0.1\%$ of the enantiomeric product detected (see ESI†).

Given that the imides **10** are generated rapidly at room temperature, the findings of Danishefsky *et al.*[8] would suggest imide formation does not proceed *via* the mixed anhydride-type intermediate, and that instead the 1,3-acyl migration occurs from the tetrahedral intermediate **4′** generating the imide directly with expulsion of silver sulfide (see Scheme 2, path b). This mechanism is also consistent with the observed lack of epimerisation, as no epimerisation-prone 'active ester' species **3′** is formed.

Use of other standard amino acid protecting groups was well tolerated, with Boc and Cbz-protected amino acids **13** and **16** undergoing the imide ligation in high yield (Scheme 3, eqn (1) and (2)). Fmoc protecting groups were not well tolerated, presumably due to the slightly basic conditions. Intriguingly, rearrangement of the carbamate-protected dipeptide imides **14a** and **17a** was observed, with significant 1,3-acyl migration generating a mixture of the initially formed imides and the isomeric species **14b** and **17b**, respectively.[12]

The imide coupling can only be considered useful in the context of peptide synthesis if regioselective hydrolysis of the imide can be achieved to generate the native amide bond. Accordingly, treatment of the Boc- and Cbz-protected dipeptide imides **14a/b** and **17a/b** under mildly basic methanolysis conditions ($NaHCO_3$, MeOH)[5e] proceeded to give the desired peptides



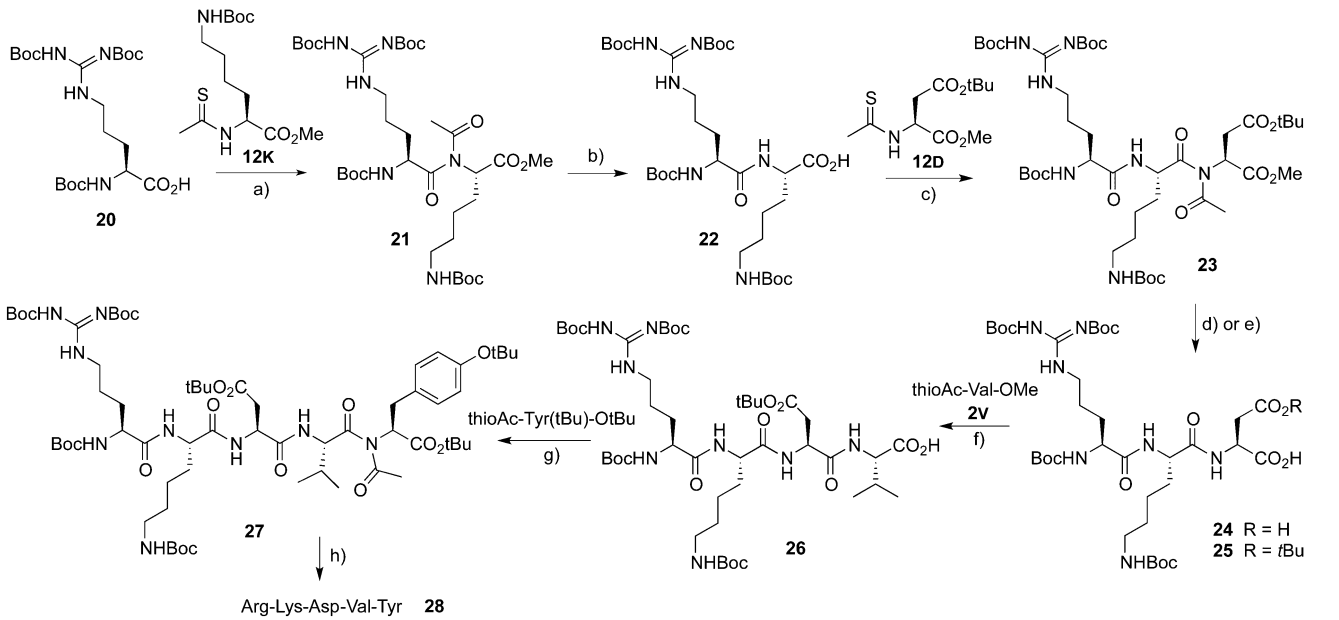
Scheme 3 Dipeptide couplings. *Reagents and conditions*; (a) Ag_2CO_3 (1.5 equiv.), CH_2Cl_2, 25 °C, 2 h. (b) $NaHCO_3$, MeOH: 73% over 2 steps for **15**, 74% over 2 steps for **18**, 62% for **19**.

15 and **18**, respectively, in good yield (Scheme 3, eqn (1) and (2)). Ultimately, for these carbamate-protected systems, solvolysis of the mixture of imides **14a/b** and **17a/b** generates a single amide product, **15** and **18**, respectively, which renders the acyl migration of the imides inconsequential to the use of this method for amide bond generation. LiOH (1 equiv.) was also effective for chemoselective imide hydrolysis (*vide infra*). Treatment of the phthaloyl-protected dipeptide imide **10GA** under similar conditions proceeded to give the desired dipeptide **19** in reasonable yield (62%) (Scheme 3, eqn (3)). Only minor amounts of the undesired imide cleavage to generate *N*-phthaloylglycine methyl ester were detected, along with trace amounts of phthaloyl-group methanolysis. The selectivity for solvolysis of the acetyl group in **10GA**, **14** and **17** over cleaving the dipeptide into its constituent amino acids is presumably due to a steric effect, with attack at the less hindered acetyl group.

To exemplify the utility of this method in peptide synthesis, we sought to generate pentapeptide thymopentin **28** through an iterative N→C coupling strategy. Thymopentin **28** is a pentapeptide fragment of the thymic hormone thymopoeitin, which has several biological actions, most notably induction of early T-cell differentiation.[13] Thymopentin corresponds to residues 32–36 of the native protein and retains most of its biological activity.[14]

The N→C synthesis of thymopentin **28** was performed as outlined in Scheme 4. While our methodology is tolerant of a range of N- and C-protecting groups, a Boc/methyl ester strategy was chosen as this would allow for a one-step concomitant imide hydrolysis/C-terminal deprotection. Accordingly, Boc-protected arginine **20** was coupled to lysine thioacetamide methyl ester **12K** to give the dipeptide imide **21** in 83% yield. Treatment of the dipeptide imide **21** with LiOH resulted in regioselective imide cleavage together with hydrolysis of the methyl ester to generate the dipeptide **22**. This sequence of thioamide coupling followed by concomitant imide and ester hydrolysis thus represents a two-step coupling–deprotection protocol suitable for peptide synthesis *via* iterative amino acid couplings.

Coupling of dipeptide **22** to aspartate thioacetamide **12D** proceeded in good yield to generate **23**. Surprisingly, it was found that treatment of tripeptide imide **23** with LiOH resulted not only in imide and methyl ester hydrolysis, but also cleavage of the side-chain *t*-butyl ester. Presumably, upon imide hydrolysis, the released amide anion undergoes intramolecular cyclisation to



Scheme 4 N→C synthesis of thymopentin. *Reagents and conditions*; (a) **12K**, Ag_2CO_3 (1.5 equiv.), CH_2Cl_2, 40 °C, 16 h, 83%; (b) LiOH (3 equiv.), 70%; (c) **12D**, Ag_2CO_3 (1.5 equiv.), CH_2Cl_2, 40 °C, 16 h, 74%; (d) LiOH (3 equiv.), dioxane/H_2O, 65% of **24**; (e) (1) $NaHCO_3$, MeOH, (2) LiOH (3 equiv.), 70% over 2 steps of **25**; (f) (1) **12V**, Ag_2CO_3 (1.5 equiv.), CH_2Cl_2, 40 °C, 16 h, 73%, (2) $NaHCO_3$, MeOH, (3) LiOH (3 equiv.), 74% over 2 steps; (g) CH_3CS-Tyr(tBu)-OtBu, Ag_2CO_3 (1.5 equiv.), CH_2Cl_2, 40 °C, 16 h, 89%; (h) (1) $NaHCO_3$, MeOH, (2) TFA, 63% over 2 steps.

generate an acyl β-lactam, which then hydrolyses to give the diacid **24**. Nevertheless, this deleterious cleavage of the *t*-butyl ester could be avoided by initial imide cleavage under the mild methanolysis conditions. Subsequent methyl ester hydrolysis with LiOH gave the tripeptide **25** with the side chain *t*-butyl ester intact. Coupling of valine thioacetamide **12V** then gave the corresponding tetrapeptide imide, which was subjected to the two-stage imide/ester hydrolysis to avoid any potential complications due to aspartimide formation, generating **26** in good yield. The tetrapeptide **26** was coupled to tyrosine *N*-thioacetamide *t*-butyl ester, with subsequent methanolysis cleaving the imide to give the protected pentapeptide **27**. Final global deprotection with TFA gave thymopentin **28** in 10% overall yield (Scheme 4), with no evidence of epimerisation or other deleterious byproducts. The synthesized peptide **28** was identical to an authentic sample by HPLC co-injection and NMR analysis (see ESI†).

In summary, we have developed a method for the coupling of amino acids and peptides with amino ester thioamides to generate peptide imides. The imides undergo regioselective hydrolysis under mild conditions to generate native peptide bonds. The use of this new method to prepare thymopentin demonstrates that our method is suitable for N→C direction peptide synthesis without epimerisation.

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