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β-Cyanomethyl-L-Ala is a masked form of L-Gln for use in solution-phase peptide synthesis.

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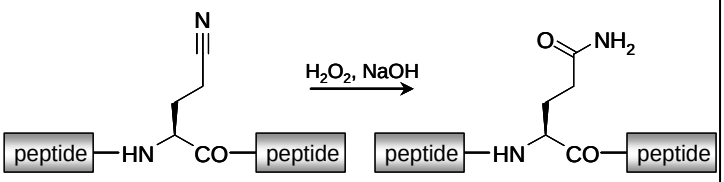

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LETTERS

S-2-Amino-4-cyanobutanoic acid (β-cyanomethyl-L-Ala) as an atom-efficient solubilising synthon for L-glutamine

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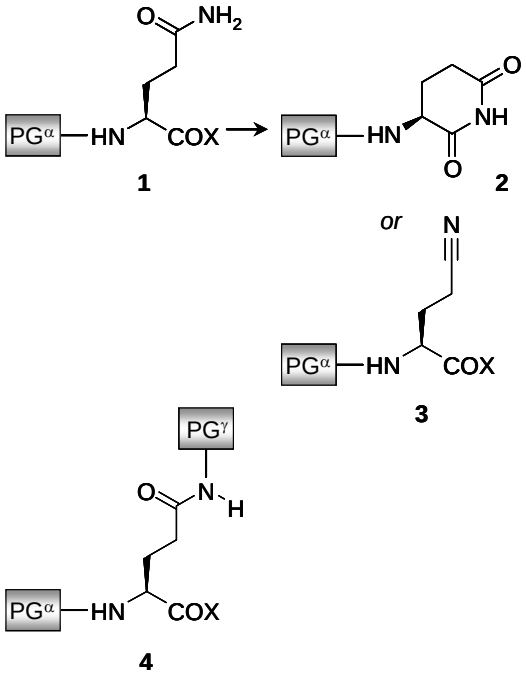
Abstract—Glutamine (Gln) is often a difficult amino acid to incorporate during solution-phase peptide synthesis, owing to poor solubility and unwanted dehydrations as side-reactions. Current approaches to solving these problems are highly atom-inefficient. N^{α}-Cbz-β-cyanomethyl-L-Ala is readily accessible by dehydration of Cbz-L-Gln. β-Cyanomethyl-L-Ala can be incorporated into short peptides easily by conventional methods. The nitrile is stable to the hydrogenolysis conditions used to remove Cbz and to acidic deprotection but is quantitatively hydrated to the γ-carboxamide of L-Gln with hydrogen peroxide. Thus β-cyanomethyl-L-Ala may represent a new, soluble, perfectly atom-efficient synthon for L-Gln. © 2012 Elsevier Science. All rights reserved

The introduction of glutamine (Gln, Q) during solution-phase synthesis of peptides is often fraught with difficulties occasioned by the presence of the primary amide in the side-chain.[1] These include limited solubility of the N^{α}-protected Gln reagent in suitable solvents for coupling, poor solubility of the product peptide (causing difficulties in purification) and unwanted reactivity of the amide.[2,3] This undesired reactivity can include cyclisation of carboxy-activated N^{α}-protected Gln **1** to N^{α}-protected α-aminoglutarimides (3-acylaminopiperidine-2,6-diones) **2** and dehydration of the γ-carboxamide to the corresponding nitrile **3** by the peptide-coupling reagent (formally "dehydrating" agents) (Scheme 1).[3] To impede these side-reactions and to aid solubility of the Gln synthon in organic solvents, many researchers have used sterically bulky protecting groups attached to the amide nitrogen atom, as in general structure **4**. Most of these protecting groups have been substituted-benzyl, substituted-diphenylmethyl (benzhydryl) or (substituted)-triphenylmethyl (trityl). These are generally synthesised from coupling of the corresponding N^{α}-protected Glu with an appropriate benzylamine, benzhydrylamine or tritylamine. Removal is normally effected by acidolysis, either as a separate step in solution-phase peptide synthesis or during release of the completed peptide from the resin during solid-phase synthesis. Examples include PG^{γ} = 4-methoxybenzyl,[4] 2,4-dimethoxybenzyl,[4,5] 2,4,6-trimethoxybenzyl,[6] tetralin-1-yl,[7] monomethoxybenzhydryl,[8] trityl,[9,10]



Scheme 1. Problematic dehydrations of Gln reagents **1** to glutarimides **2** and nitriles **3** reported during peptide couplings with side-chain-unprotected Gln and structures of N^{γ}-protected Gln coupling reagents **4**.

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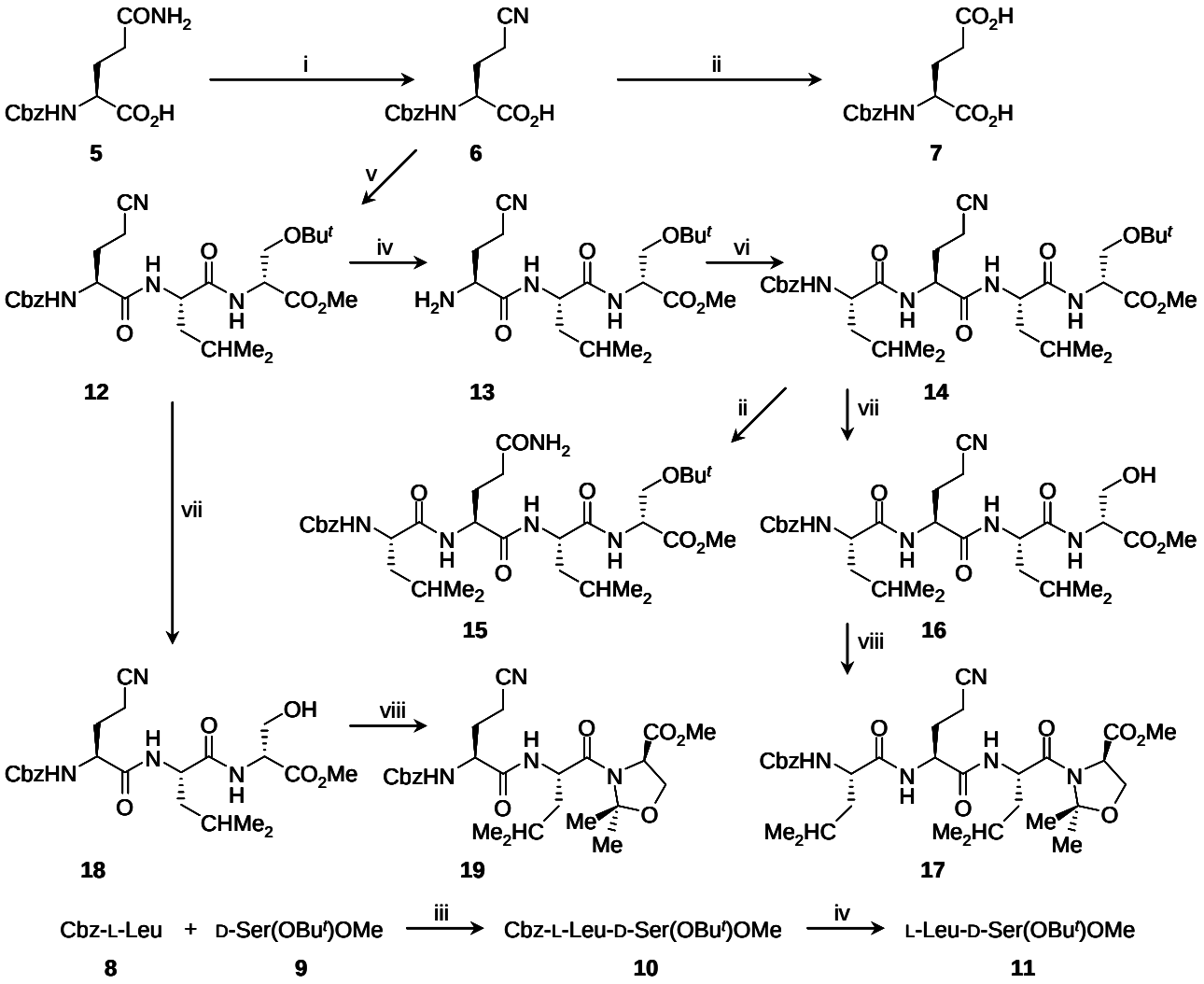
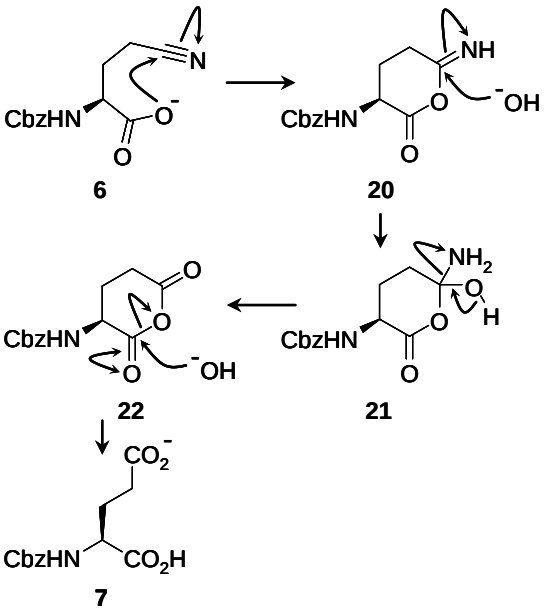


Scheme 2. Examples of the use of the *S*-2-(Cbz-amino)-4-cyanobutanoic acid as a synthon for L-Gln in solution-phase peptide synthesis. *Reagents and conditions*: i, DCC, pyridine, 92%; ii, aq. H_2O_2, NaOH, 100% (**7**), 90% (**15**); iii, PyBOP, Et_3N, CH_2Cl_2, (95%); iv, H_2, Pd/C, MeOH, 100% (**11**), 100% (**13**); v, **11**, HATU, $^iPr_2NEt$, CH_2Cl_2, 77%; vi, **8**, PyBOP, Et_3N, CH_2Cl_2, 75%; vii, CF_3CO_2H, CH_2Cl_2, 100% (**16**), 100% (**18**); viii, $Me_2C(OMe)_2$, TsOH, CH_2Cl_2, reflux, 22% (**17**), 50% (**19**).

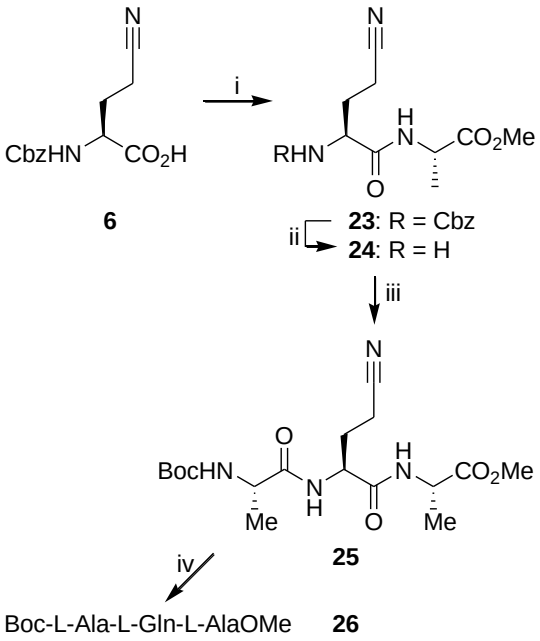
and the monomethyltrityl.[11,12] 2-Nitrobenzyl has also been used as a photo-cleavable protection.[13] Each of these has the disadvantage of being atom-inefficient, in that a bulky protecting group necessarily contains many atoms, and generating involatile side-products upon removal, which have to be separated during purification of the target peptide. It would thus be useful to have available a protection tactic for the side-chain of Gln which achieves the required solubility and lack of unwanted reactivity and which is highly atom-efficient, not requiring removal of involatile side products as the Gln side-chain is regenerated.

In the present work, we have discovered that the hitherto "unwanted" dehydration of the side-chain of Gln to the corresponding nitrile can be exploited for atom-efficient protection. Ressler and Ratzkin have described[14] a useful dehydration of the side-chain primary carboxamide of N^{α}-CbzGlnOH **5** to the corresponding nitrile **6** (Scheme 2) using dicyclohexylcarbodiimide in pyridine and we were able to reproduce this in excellent yield, after modification. Webber *et al*. dehydrated BocGlnOMe with $POCl_3$ and pyridine and used the product to introduce β-cyanomethyl-L-alanine (2-amino-4-cyanopentanoic acid) into target peptides as a lipophilic isostere of Gln.[15] β-Cyanomethyl-L-alanine has also been used as an unnatural amino acid in the pharmacophores of bioactive synthetic peptides[16,17] and a precursor to peptides containing tetrazoles in their side chains.[18] However, rehydration of this side chain to Gln has not yet been explored.

We have previously demonstrated that benzonitriles are readily hydrated to benzamides under mild conditions, while retaining other chemically-sensitive peripheral functional groups, by the use of hydrogen peroxide under mild basic conditions.[19] However, application of this procedure to compound **6** led, quantitatively, to the carboxylic acid CbzGlu **7**. It is likely that the α-carboxylate participates as an intramolecular nucleophile, out-competing the intermol-



Scheme 3. Proposed mechanism for hydrolysis of **6** to **7** with the hydroperoxide ion.



Scheme 4. Further example of the use of *S*-4-cyanobutanoic acid as a synthon for L-Gln in solution-phase peptide synthesis; compatibility with NBoc protection. *Reagents and conditions*: i, L-AlaOMe.HCl, PyBOP, Pr^i_2NEt, DMF, 87%; ii, H_2, Pd/C, MeOH; iii, Boc-L-AlaOH, Pr^i_2NEt, HATU, DMF, 64% from **23**; iv, aq. H_2O_2, NaOH, MeOH, 0°C; 23% .

ecular attack of hydroperoxide ion, as shown in Scheme 3. This result indicates that the strategy of hydrating β-cyanomethyl-L-Ala residues at the C-terminus of synthetic peptides will not be useful.

In contrast, β-cyanomethyl-L-Ala is a useful synthetic equivalent of Gln within the peptide chain. As shown in Scheme 2, Cbz-L-Leu-D-Ser(OBu^t)OMe **10** was prepared in the usual way by coupling Cbz-L-Leu **8** with D-Ser(OBu^t)-OMe **9** using PyBOP. Cbz was then removed quantitatively by hydrogenolysis, affording the dipeptide L-Leu-D-Ser-(OBu^t)OMe **11**. *N*-Cbz-β-cyanomethyl-L-Ala **6** coupled very effectively with this dipeptide **11** by the HATU method to provide the protected tripeptide **12**,[20] demonstrating that it can be attached easily to growing chains. The Cbz group was then removed by hydrogenolysis under standard conditions (H_2, Pd/C) to give **13** quantitatively, with no evidence of any competing reduction of the nitrile.[21] Coupling with Cbz-L-LeuOH **8** then led to the protected tetrapeptide **14** in good yield,[22] showing that peptide couplings to the N-terminus of β-cyanomethyl-L-Ala are efficient. Tetrapeptide **14** contains β-cyanomethyl-L-Ala as a masked Gln. The Gln was revealed quantitatively by mild hydration with hydrogen peroxide under basic conditions, forming **15**, with no evidence for over-hydrolysis to the carboxylic acid (Glu).[23] Thus, within a peptide sequence, regeneration of the carboxamide is feasible and efficient. To test the stability of the nitrile under acidic deprotection conditions, **14** was treated with trifluoroacetic acid, unmasking the Ser side chain in **16**. From here, acid-catalysed condensation with 2,2-dimethoxypropane furnished the tetrapeptide **17**, which contains both the C-terminal D-Dmo residue (which can be considered as a masked D-Ser and as an analogue of D-Pro) and the β-cyanomethyl-L-Ala (masked Gln). Analogously, the side-chain Bu^t protection was removed from the D-Ser of the shorter peptide **12**, giving **18**,[24] prior to formation of the D-Dmo-containing tripeptide **19**.[25]

Scheme 4 gives further demonstrations of the compatibility and use of β-cyanomethyl-L-Ala in solution-phase peptide synthesis. Cbz-β-cyanomethyl-L-AlaOH **6** was coupled to L-alanine methyl ester by the PyBOP method to give protected dipeptide **23**.[26] In this case, the hydrogenolytic cleavage of Cbz had to be monitored carefully, as overreaction gave traces of the product of reduction of the nitrile (L-Orn-L-AlaOMe) and of the diketopiperazine cyclo-L-(β-cyanomethyl-Ala)-L-Ala. Immediate coupling of the amine **24** with Boc-L-AlaOH by the HATU procedure gave masked tripeptide **25**.[27] Careful hydration of the nitrile with hydroperoxide ion was achieved without disruption of the N-Boc protection, generating Boc-L-Ala-L-Gln-L-AlaOMe **26**.[28]

In this *Letter*, we introduce β-cyanomethyl-L-Ala (*S*-2-amino-4-cyanobutanoic acid) as a potentially useful and perfectly atom-efficient synthetic equivalent of L-Gln in solution-phase peptide synthesis. N^{α}-Cbz-β-cyanomethyl-L-Ala **6** is generated in high yield from Cbz-L-Gln **5**. The Cbz protection can be removed selectively by hydrogenolysis, under conditions which preserve the nitrile completely. The nitrile is also stable to the acidic conditions required for removal of Boc and OBu^t protecting groups and for condensations and to the basic conditions for removal of Fmoc. It is, however, rapidly and quantitatively hydrated to the primary carboxamide of L-Gln. Thus we propose β-cyanomethyl-L-Ala as a synthon for L-Gln; complete characteris-

ation of its uses and limitations will be presented in a later full paper.

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20. **Synthesis of 12.** Compound **6** (900 mg, 3.43 mmol) and L-Leu-D-Ser(OBu^t)-OMe **11** (1.00 g, 3.43 mmol) were stirred with 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (1.43 g, 3.77 mmol) and Pr$^i_2$NEt (885 mg, 6.86 mmol) in CH_2Cl_2 (80 mL) for 20 h. Washing (H_2O (50 mL), aq. citric acid (5%, 50 mL)), aq. $NaHCO_3$ (5%, 100 mL), H_2O (50 mL)), drying, evaporation and chromatography (EtOAc, silica gel) gave **12** (1.14 g, 77%) as a white powder: mp 95-96 °C.
21. **Synthesis of 13.** Compound **12** (820 mg, 1.54 mmol) was stirred vigorously under H_2 with Pd/C (10%, 80 mg) in MeOH (80 mL) for 16 h. Filtration (Celite®) and evaporation gave **13** (640 mg, quant.) as a pale yellow oil.
22. **Synthesis of 14.** Cbz-L-LeuOH **8** (210 mg, 0.80 mmol) and **13** (320 mg, 0.80 mmol) were stirred with benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (500 mg, 0.96 mmol) and Et_3N (323 mg, 3.20 mmol) in CH_2Cl_2 (15 mL) for 2 d. Washing (H_2O (20 mL), aq. citric acid (5%, 20 mL)), aq. $NaHCO_3$ (5%, 20 mL), H_2O (40 mL)), drying, evaporation and chromatography (petroleum ether / EtOAc 7:3) gave **14** (390 mg, 75%) as a white powder: mp 152-154 ºC.
23. **Synthesis of 15.** Aq. H_2O_2 (35%, 0.30 mL) was added to **14** (20 mg, 30 μmol) in aq. NaOH (0.5 M, 2.7 mL), followed by aq. NaOH (2.0 M, 0.2 mL). The mixture was stirred for 1 h at room temperature. The mixture was cooled to 0 ºC and the pH was adjusted to 2.0 with aq. HCl (1.0 M). Extraction (CH_2Cl_2 (10 mL, thrice)), drying and evaporation gave **15** (18.8 mg, 90%) as a white powder: mp 193-194 °C.
24. **Synthesis of 18.** Compound **12** (200 mg, 0.37 mmol) was stirred with CF_3CO_2H (2.0 mL) and CH_2Cl_2 (2.0 mL) for 2 h. Evaporation gave **18** (220 mg, quant.) as a yellow gum.
25. **Synthesis of 19.** Compound **18** (220 mg, 0.46 mmol) was boiled under reflux with 2,2-dimethoxypropane (240 mg, 2.3 mmol) and TsOH (9.6 mg, 5 μmol) in CH_2Cl_2 (5.0 mL) under N_2 for 19 h. Evaporation and chromatography (petroleum ether / EtOAc 4:1) gave **19** (120 mg, 50%) as a yellow oil.
26. **Synthesis of 23**. Compound **6** (3.06 g, 11.7 mmol) was stirred with PyBOP (9.10 g, 17.5 mmol), Pr$^i_2$NEt (8.89 g, 68.9 mmol) and TsOH (9.6 mg, 5 μmol) in CH_2Cl_2 (5.0 mL) under N_2 for 19 h. Evaporation and chromatography (petroleum ether / EtOAc 4:1) gave **19** (120 mg, 50%) as a yellow oil.
27. **Synthesis of 25.** Compound **23** (357 mg, 1.03 mmol) in MeOH (25 mL) was stirred vigorously under H_2 with Pd/C (10%, 36 mg) for 2 h. Filtration (Celite®) and evaporation gave crude **24**. This material was stirred with Boc-L-AlaOH (395 mg, 2.1 mmol), Pr$^i_2$NEt (533 mg, 4.1 mmol) and HATU (822 mg, 2.2 mmol) in DMF (10 mL) for 2 h. The evaporation residue, in EtOAc (50 mL), was washed (aq. $NaHCO_3$ (50 mL), brine (25 mL)). Drying, evaporation and chromatography (petroleum ether / EtOAc 1:1 → EtOAc) gave **25** (252 mg, 64%) as a white solid: mp 175-176 °C; $[\alpha]^{18}_D$ -24.6 (*c* = 0.80, DMF).
28. **Synthesis of 26.** Compound **25** (1.00 g, 2.6 mmol) was dissolved in MeOH (50 mL) and the mixture was cooled to 0 °C. Aq. H_2O_2 (35%, 1.6 mL, 18 mmol) was added, followed by aq. NaOH (1.0 M, 2.6 mL, 2.6 mmol), and the mixture was stirred at 0 °C for 6 h. Aq. Na_2SO_3 (20%, 30 mL) was added. The evaporation residue, in EtOAc (100 mL), was washed (brine (50 mL)). Drying, evaporation and chromatography (EtOAc → EtOAc / MeOH 4:1) gave **26** (243 mg, 23%) as a white solid: mp 193-194 °C; $[\alpha]^{18}_D$ -26.4 (*c* = 0.85, DMF).