ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2014, **50**, 1259

Received 7th November 2013,
Accepted 26th November 2013

DOI: 10.1039/c3cc48509a

www.rsc.org/chemcomm

Solution-phase-peptide synthesis *via* the group-assisted purification (GAP) chemistry without using chromatography and recrystallization†

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The solution phase synthesis of *N*-protected amino acids and peptides has been achieved through the Group-Assisted Purification (GAP) chemistry by avoiding disadvantages of other methods in regard to the difficult scale-up, expenses of solid and soluble polymers, *etc.* The GAP synthesis can reduce the use of solvents, silica gels, energy and manpower. In addition, the GAP auxiliary can be conveniently recovered for re-use and is environmentally friendly and benign, and substantially reduces waste production in academic labs and industry.

Peptides and peptidomimetic drugs are involved in nearly all disciplines of chemical and biomedical sciences.[1–5] The search for environmentally friendly synthetic approaches to these products for academic research and pharmaceutical production has been pursued for over five decades.[6–11] A notable accomplishment in peptide synthesis is shown by the solid-phase-peptide synthesis (SPPS) invented by the late Nobel Laureate, Bruce Merrifield, in the 1980's.[12–13] This milestone discovery can overcome shortcomings of traditional solution-phase-peptide synthesis in regard to tedious purification, consumption of large amounts of materials including coupling reagents, solvents and silica gels, and can reduce waste generation; it has also greatly accelerated peptide and protein research. SPPS has also been imposing a huge impact on general chemical synthesis for drug discovery and development, particularly, on combinatorial screening.[13] Since then several synthetic protocols have been developed to complement SPPS so as to minimize the difficulty of scale-up, the use of excess amounts of reagents and expensive resins for coupling reactions, particularly for synthesizing longer peptides.[14–21] In this regard, an alternative method was developed by using soluble polymers as the templates for coupling of amino acid residues.[22,23] This method enables the peptide synthesis to be performed in the solution phase but the work-up in a solid-phase manner, or, through convenient extractions. However, the latter method needs soluble polymers of high molecular weights, which makes it inconvenient to produce large amounts of peptides of much lower molecular weights than polymeric templates. In addition, it often needs long periods to generate solid or crystalline products by carefully controlling solidification/crystallization conditions.

Recently, our labs have established the GAP (Group-Assisted Purification) chemistry concept through the design and synthesis of new reagents that were attached with unique achiral and chiral auxiliaries.[24–31] By using GAP technology, organic synthesis can be performed efficiently and stereochemically without the use of traditional purification methods such as chromatography, recrystallization, *etc.* In fact, the stereoisomeric products can be obtained simply by washing the crude mixtures with inexpensive petroleum solvents or co-solvents. Therefore, it can substantially reduce the use of starting materials, silica gels, energy, manpower, *etc.* In addition, achiral and chiral auxiliaries can be conveniently recovered for re-use, and they can often be quantitatively recovered *via* a one-time extraction with *n*-butanol. It is believed that the GAP chemistry concept will have a huge impact on chemical synthesis and medicinal fields. In this report, we would like to disclose that the GAP chemistry strategy can advance peptide synthesis, which has advantages of both solid-phase-peptide synthesis (SPPS) and liquid-phase synthesis of peptides by avoiding their shortcomings in regard to the factors mentioned above.[22,23]

Amino acid residues are basic building blocks for the synthesis of peptides and proteins. We first explored if individual amino acids can be protected *via* the GAP chemistry protocol. *N*,*N*′-Di-(1-naphthylmethylene)-1,2-cyclohexyldiamino *N*-phosphonyl chloride A-1 was employed for the reactions with L-Pro-OBn·HCl, D-Ala-OMe·HCl and L-Phe-OMe·HCl. To a solution of *N*-phosphonyl chloride in dichloromethane triethylamine (2.5 eq.), 4 Å MS and amino ester were added; the resulting mixture was stirred at 90 °C overnight for complete consumption of major starting materials. The pure protected amino acid esters of the above three products were obtained *via* the GAP work-up washing with hexane–DCM co-solvents by avoiding the use of column chromatography or

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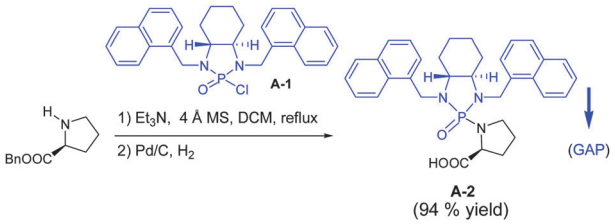
† Electronic supplementary information (ESI) available: Spectroscopic spectra of all pure products shown in Schemes S1–S3 and Fig. S1. See DOI: 10.1039/c3cc48509a

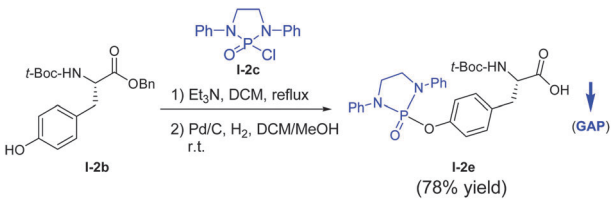


Scheme 1 GAP protection of L-proline benzyl ester and its deprotection.

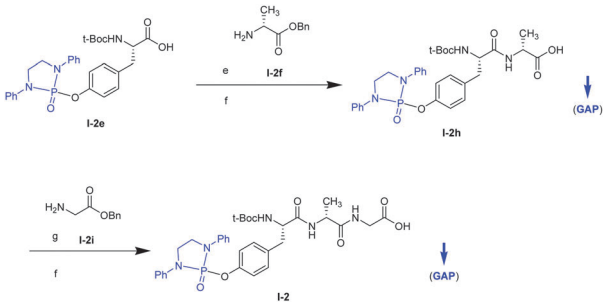
recrystallization to give yields of 96%, 92% and 86%, respectively. For this protection experiment, 4 Å MS was necessary for achieving higher yields. Interestingly, the protection of secondary amino acid ester (L-Pro) occurred more smoothly to give better yields than other primary amino cases (Scheme 1). The following catalytic hydrogenation afforded corresponding *N*-phosphonyl amino acids with free carboxylic acid groups.[32] Our attention was next turned to the protection of the hydroxy group of Tyr which is among the most important aromatic amino acids[5,33] so as to explore if the GAP chemistry strategy is suitable for peptide synthesis. The simpler achiral *N*-phosphonyl chloride was utilized for the protection which was followed by catalytic hydrogenation to afford corresponding *N*-phosphonyl tyrosine (Scheme 2). At this initial stage, this concise and achiral protection is being continued for peptide synthesis, although the GAP case of using chiral ones is also showing promising potential in this laboratory.

The new GAP strategy is presented by the synthesis of biphalin peptides that belong to the opioid pain killer series.[33–37] The original biphalin is a highly potent analgesic candidate but shows low selectivity between δ and μ receptors.[35–39] Its structural modifications are anticipated to generate new pain killers of high potency and selectivity so as to reduce unwanted side effects. The use of a phosphoric acid moiety for modifying peptides would meet the above purpose. In fact, this strategy has not been well documented yet. The present GAP peptide synthesis will concisely generate a series of new biphalin derivatives and greatly accelerate opioid peptide research.

The GAP synthesis of dipeptide and tripeptide precursors to biphalins is represented by the example shown in Scheme 3. The coupling reactions were conducted under standard conditions of using TBTU and Et_3N in DCM at room temperature.[32] The catalytic hydrogenation to cleave the benzyl group gives *O*-phosphonyl amino acid for the next coupling reaction. The individual *N*-*t*-Boc amino acid benzyl esters of D-Ala, Gly and Phe were prepared by following literature procedures.[32] The dipeptide bridge precursors, **I-3a** to **3d** and **I-4a** to **4d**, were also synthesized by following known methods in standard coupling systems.[38]



Scheme 2 GAP protection of L-tyrosine benzyl ester and its deprotection.



(e) TBTU, Et_3N, DCM, rt overnight; (f) 10% Pd/C, H_2, DCM/MeOH, rt 12 h; (g) TBTU, Et_3N, DCM, rt overnight; (h) 10% Pd/C, H_2, DCM/MeOH, rt 12 h.

Scheme 3 GAP synthesis of dipeptides and tripeptides.

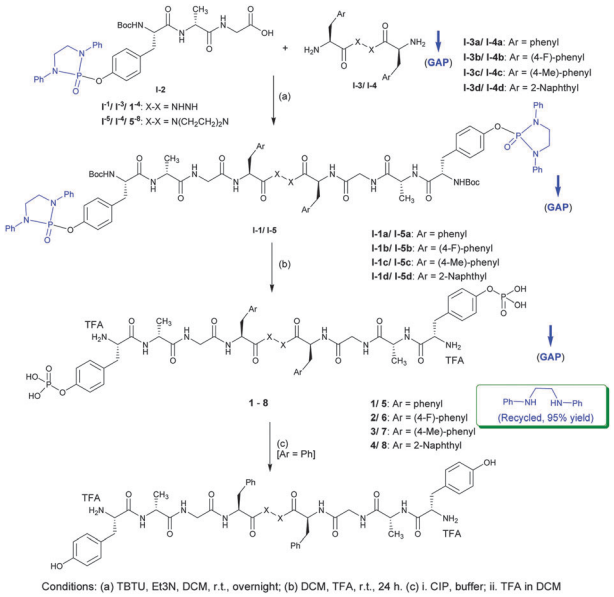
It should be pointed out that that the GAP purification was successfully applied for the preparation of **I-3a** to **3d**. In this preparation, the crude bis-coupling products were washed with a co-solvent of EtOAc–hexanes (1 : 2, v/v) to give pure **I-3a** to **3d** as white solids. Similarly, the crude products of **I-4a** to **4d** obtained *via* the GAP procedure were washed with a co-solvent of dichloromethane–hexanes (1 : 100, v/v) to give pure **I-4a** to **4d** as white solids as well.

N,*N*′-Di-phenyl-1,2-ethyldiamino *O*-phosphonyl attached biphalins, **I-1**, were synthesized by reacting tripeptide **I-2** (1.0 eq.) and dimeric dipeptide **I-3** (0.5 eq.) in dry dichloromethane (DCM) in the presence of triethyl amine (Et_3N) (1.1 eq.) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (1.1 eq.) as the coupling reagent.[12] The reaction mixture was cooled to 0 °C before TBTU was added and kept stirring for 2 hours at this temperature. The coupling reaction was completed after being stirred at room temperature overnight. The reaction mixture was diluted to double volume by DCM and washed with H_2O, 0.5 M HCl, aq $NaHCO_3$(sat.) and brine in order (Scheme 4).

The new *O*-phosphoryl biphalin **1** was generated by treating **I-1** with trifluoroacetic acid (TFA) in DCM at r.t. for 24 hours as monitored by ^{31}P NMR. After the cleavage was finished, the resulting mixture was evaporated before H_2O and DCM (1 : 1, v/v) were added. The resulting mixture was extracted with DCM five times to completely remove the *N*,*N*′-biphenylethylenediamine auxiliary from the aqueous phase. Evaporating the aqueous phase to dryness afforded pure *O*-phosphoryl biphalin **1** as a white solid in 88% yield. It is noteworthy that the *N*,*N*′-biphenylethylenediamine auxiliary can be recovered from DCM at a 95% recovery rate.

Other *O*-phosphoryl biphalins **2–8** were conveniently obtained under the same conditions and worked-up by following the GAP washing without the use of column chromatography or re-crystallization. They were obtained as white solids in good to excellent yields of 78% to 92%.

After we successfully synthesized the new biphalin analogs with the phosphoryl moiety attached on the tyrosine ring that would lead to interesting binding and biological profiles in future, we further performed mild cleavage to give free biphalins.[32] We found that the treatment of *O*-phosphonyl biphalins with calf intestinal phosphatase (CIP) in a buffer solution of pH 7–8 at room temperature afforded free biphalin peptide in 87% of chemical yield.



Scheme 4 GAP synthesis of biphalins.

The Group-Assisted Purification (GAP) strategy has been successfully utilized for the solution phase synthesis of *N*-protected amino acids and peptides by avoiding the use of traditional purification protocols such as chromatography and recrystallization. In the present GAP synthesis, pure products have been obtained simply by washing the crude mixtures with inexpensive petroleum solvents or co-solvents to give good to high yields. In addition, the GAP auxiliary can be conveniently recovered for re-use.

This work is supported by NIH (R33DA031860, USA), Robert A. Welch Foundation (D-1361, USA) and Jiangsu Innovation Team Program (P. R. China).

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