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New methodology for high throughput solution-phase synthesis: affinity purification by using crown ether and ammonium ion interaction

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Abstract

A new method for affinity purification of synthetic compounds by using crown ether–ammonium ion interaction and its application to the synthesis of several peptides and heterocycles are described. The desired compounds possessing the crown ether (32-crown-10) moiety were readily isolated from the reaction mixture by the following procedure. After each reaction cycle, the reaction mixture was applied to the aminomethylated polystyrene column (trifluoroacetic acid form). The compound possessing the crown ether was selectively adsorbed on the column, whereas other impurities without the crown ether such as excess reagents and byproducts were washed off. Subsequent desorption by Et_3N or CH_2Cl_2:MeOH (1:1) afforded the desired compound with high purity. This new strategy is expected to be useful, for, in particular, multiple parallel synthesis and combinatorial library preparation.

Keywords: affinity purification; ammonium ion; crown ether; high throughput synthesis; combinatorial chemistry.

The solid-phase strategy has been a strong tool for high throughput synthesis and combinatorial chemistry, since excess reagents and byproducts can be quickly removed by filtration. However, there are several disadvantages to solid-phase synthesis: (i) some reaction conditions in solution are not compatible with reactions on solid-supports; (ii) the reaction rates on solid-supports are generally lower than those in solutions; and (iii) monitoring of the reaction is difficult when using simple and routine methods such as TLC and HPLC.

Affinity column chromatography, by using specific interactions such as metal chelation with an oligohistidine tag, allows rapid purification of the desired recombinant proteins.[1] The 'tag' methodology should also be applicable to organic synthesis by using chemically stable tags and enable rapid separation of the desired compounds from reaction mixtures. The reaction can be carried out in solution and hence

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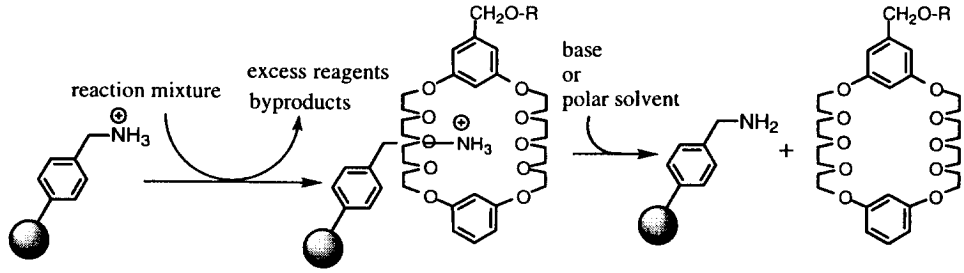
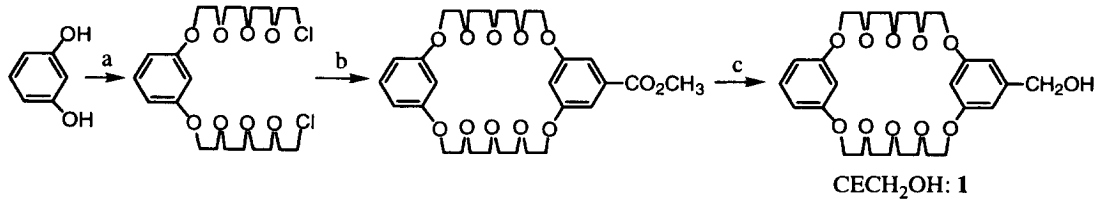
monitored by TLC or HPLC.[2–6] We describe here a novel 'tag' methodology for rapid separation of the desired compounds by using interaction between a crown ether and ammonium ion (Scheme 1).



Scheme 1.

5-(Hydroxymethyl)-1,3-phenylene-*m*-phenylene-32-crown-10 ($CECH_2OH$: **1**) prepared according to Scheme 2[7] was used as a tag.[8] Aminomethylated polystyrene resins [trifluoroacetic acid (TFA) form] were used as a column stationary phase.[9] A highly cross-linked macroporous resin [ArgoPore™–NH_2 (amine loading: 1.39 mmol/g)[10]] gave better separation than the usual 1% cross-linked gel-type resin. $CECH_2OH$ **1** did not bind to an anion exchange resin having a quaternary ammonium group. Nonpolar solvents such as CH_2Cl_2 and $CHCl_3$ are suitable as eluents for binding of the crown ether. Polar solvents such as methanol, ethanol, and DMF desorb $CECH_2OH$ **1** from the column, since solvation by polar solvents may decrease the interaction of the crown ether with the ammonium ion, and some parts of TFA were washed out of the column. The use of acetone and ethyl acetate should be avoided, since they may react with the primary amino group.



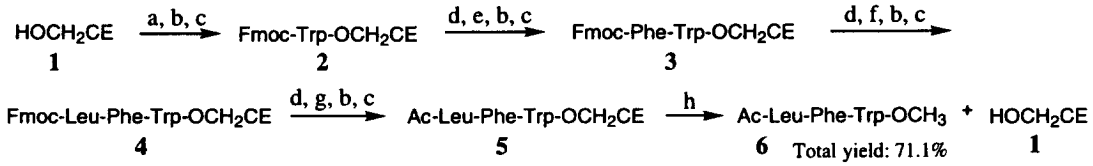
$CECH_2OH$: **1**

Scheme 2. (a) $ClCH_2(CH_2OCH_2)_3CH_2Cl$ (10 equiv.), NaH in DMF, 90°C, 2 h, 67.1%; (b) methyl 3,5-dihydroxybenzoate (1 equiv.), K_2CO_3 in DMF, 115°C, 6 h, 35.1%; (c) LAH (2 equiv.) in THF, rt, 10 h, 90.5%

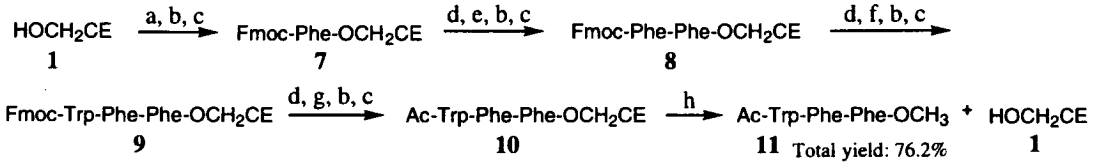
After each reaction, the reaction mixture was applied to the column possessing an ammonium ion. The desired compound having the crown ether moiety was selectively retained in the column, whereas other compounds without the tag were washed off. The desired compound with the crown ether tag was then eluted with diluted Et_3N solution in CH_2Cl_2 or polar solvents as eluents.[11,12]

This methodology was first applied to the synthesis of tripeptides **6** and **11** (Schemes 3 and 4). All the reactions were carried out in solution at room temperature. As shown in Scheme 3, $CECH_2OH$ was esterified with Fmoc-Trp-OH by using 2 equiv. of diisopropylcarbodiimide (DIC) and a catalytic amount of 4,4-dimethylaminopyridine (DMAP) in CH_2Cl_2. The reaction mixture was then applied to an ArgoPore™–$NH_3^+ \cdot CF_3CO_2^-$ column. Fmoc-Trp-OH, DIC, DMAP, and *N,N'*-diisopropylurea were removed by elution with CH_2Cl_2, whereas the desired **2** was adsorbed on the resin. Compound **2** was then eluted with 5% Et_3N in CH_2Cl_2. The eluate was washed with water to remove $CF_3COOH \cdot Et_3N$ and then concentrated in vacuo to give **2**. The Fmoc group of **2** was then removed by 5% piperidine in DMF for 8 min and the solution was concentrated in vacuo. Fmoc-Phe-OH was then coupled by the DIC method to give dipeptide **3**, which was purified by the same method. Tripeptide **4** was obtained by removal of the Fmoc group, coupling with Fmoc-Leu-OH, and the affinity purification. Removal of the Fmoc group of **4** followed by *N*-acetylation afforded **5**. The crown ether moiety of **5** was then

removed by transesterification to give **6**. Tripeptide **6** and the crown ether **1** were not separated by this affinity separation, since **6** was also adsorbed on the resin in CH_2Cl_2 though the nature of the interaction is unknown. Purification by silica-gel column chromatography afforded tripeptide **6** in a total yield of 71.1% from **1**.



Scheme 3. (a) Fmoc-Trp-OH (1.25 equiv.), DIC (2 equiv.), DMAP (cat.) in CH_2Cl_2; 30 min; (b) ArgoPore™–$NH_3^+ \cdot CF_3CO_2^-$ column; (c) elution by Et_3N in CH_2Cl_2; (d) 5% piperidine in DMF, 8 min; (e) Fmoc-Phe-OH (1.25 equiv.), DIC (2 equiv.) in CH_2Cl_2, 30 min; (f) Fmoc-Leu-OH (1.25 equiv.), DIC (2 equiv.) in CH_2Cl_2, 30 min; (g) Ac_2O, Et_3N in CH_2Cl_2, 1 h; (h) 0.2 M MeONa/MeOH, 10 min

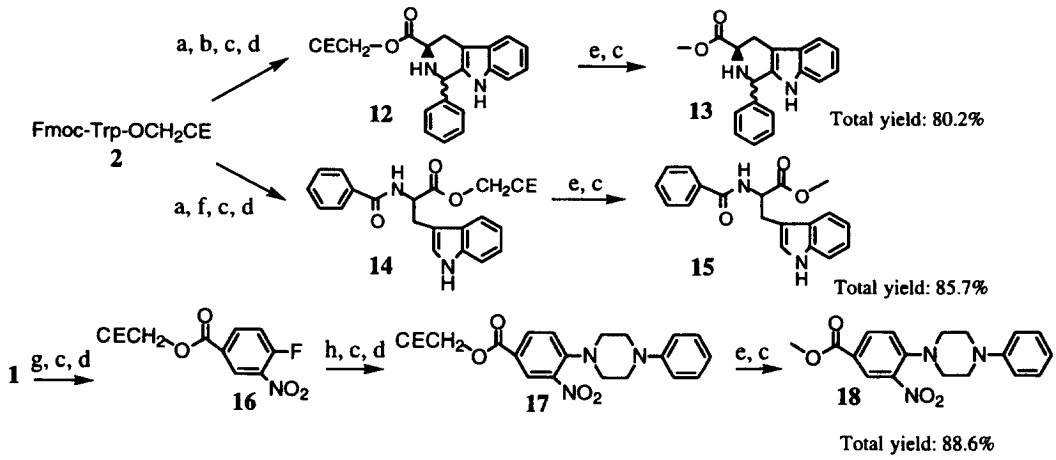


Scheme 4. (a) Fmoc-Phe-OH (1.25 equiv.), DIC (2 equiv.), DMAP (cat.) in CH_2Cl_2, 30 min; (b) ArgoPore™–$NH_3^+ \cdot CF_3CO_2^-$ column; (c) elution with CH_2Cl_2:MeOH (1:1); (d) 5% piperidine in DMF, 8 min; (e) Fmoc-Phe-OH (1.25 equiv.), DIC (2 equiv.) in CH_2Cl_2, 30 min; (f) Fmoc-Trp-OH (1.25 equiv.), DIC (2 equiv.) in CH_2Cl_2, 30 min; (g) Ac_2O, Et_3N in CH_2Cl_2, 1 h; (h) 0.2 M MeONa/MeOH, 10 min

Since elution of the crown ethers with Et_3N required a washing process to remove $CF_3COOH \cdot Et_3N$, we then examined the elution with CH_2Cl_2:MeOH (1:1) in the synthesis of tripeptide **11** (Scheme 4). Compounds **7**, **8**, **9**, and **10** were isolated by the following procedure: adsorption by the aid of the crown ether on the column by using CH_2Cl_2 as an eluent and desorption with CH_2Cl_2:MeOH (1:1) from the column. The desired synthetic intermediates were obtained just by vacuum concentration of the eluates. Tripeptide **11** was obtained by a much simpler procedure than the previous one in 76.2% from **1** after purification by silica-gel column chromatography.

Several heterocycles were synthesized by using this method as shown in Scheme 5. After removal of the Fmoc group of **2**, reaction with benzaldehyde in 1% TFA/CH_2Cl_2 gave the β-carboline derivative **12**,[13] which was purified in the same manner as the preparation of **7**. The crown ether moiety of **12** was then removed by transesterification. After usual work-up, the mixture of **13** and **1** in CH_2Cl_2 was applied to the ArgoPore™–$NH_3^+ \cdot CF_3CO_2^-$ column. Compound **13** passed through the column, whereas crown ether **1** was adsorbed on the column. After evaporation, **13** was obtained in 80.2% yield. Crown ether **1** was then recovered by elution with CH_2Cl_2:MeOH (1:1). *N*-Benzoyl tryptophan methyl ester **15** was prepared via the same purification procedure.

Diarylpiperazine **18** was prepared via aromatic nucleophilic substitution.[14,15] 1-Methyl-2-pyrrolidone (NMP), which reduces the interaction of the crown ether and the ammonium ion, was used as a solvent at the nucleophilic substitution step. After excess *N*-phenylpiperazine was quenched with Ac_2O, the NMP solution was diluted with CH_2Cl_2 and then subjected to the affinity separation. Purification of diarylpiperazine **17** was thus effected in the same manner without the additional step for the removal of NMP. Transesterifaction and isolation by using ArgoPore™ column gave **18** in total 88.6% yield.



Scheme 5. (a) 5% Piperidine in DMF, 8 min; (b) PhCHO (2 equiv.), 1% TFA in CH_2Cl_2, 24 h; (c) ArgoPore™–$NH_3^+ \cdot CF_3CO_2^-$ column; (d) elution with CH_2Cl_2:MeOH (1:1); (e) 0.2 M MeONa/MeOH, 10 min; (f) PhCOCl (2 equiv.), TEA in CH_2Cl_2, 1 h; (g) 4-fluoro-3-nitrobenzoic acid (1.25 equiv.), DIC (2.5 equiv.), DMAP (cat.) in CH_2Cl_2; (h) i: *N*-phenylpiperazine (2 equiv.) in NMP; ii: Ac_2O

Table 1
The purity of compounds

Compounds	Purity (%)	Compounds	Purity(%)
6	92	**14**	94
10	86	**15**	96
11	93	**17**	97
13	83	**18**	94

The structures of all intermediates and final products were confirmed by NMR and/or MS spectrometry. The purity of some intermediates and all the final products without any further purification are summarized (Table 1) as checked by HPLC (detection at 250 nm).

By using appropriate linkers, the present method can be applied to the synthesis of many kinds of organic compounds. Purification of the desired compounds by the present method is much faster than the usual work-up, i.e., extraction, concentration, and silica-gel column chromatography. This technique will allow an efficient way in multiple parallel synthesis and combinatorial library preparation.

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9. The crown ether **1** was not retained in the column of ArgoPore™–NH_2 acetic acid form but was retained in that of the formic acid form. TFA is, however, more effective.
10. Commercially available from Argonaut Technologies, San Carlos, California: http://www.argotech.com.
11. The resin was recovered by washing with methanol, CH_2Cl_2, and then TFA solution in CH_2Cl_2 after each cycle.
12. Typical procedure: 8 ml of CH_2Cl_2 and 1.5 ml of TFA were added to 4.0 g of ArgoPore™–NH_2 (1.39 mmol/g), and the mixture was shaken at rt for 2 h, and transferred into a glass column. The resulting resin was washed with CH_2Cl_2 (50 ml). Ester **16** prepared from crown ether **1** (51 mg, 0.0901 mmol) and 4-fluoro-3-nitrobenzoic acid was dissolved in NMP (1 ml). 1-Phenylpiperazine (41 μl, 0.27 mmol) was added to the solution. The mixture was stirred at rt for 1 h and diluted with 2 ml of CH_2Cl_2. After 3 drops of Ac_2O were added, the mixture was further diluted with 8 ml of CH_2Cl_2 and then applied to the ArgoPore™–$NH_3^+ \cdot CF_3CO_2^-$ column. After undesired compounds were washed off with CH_2Cl_2, the desired **17** was eluted with MeOH:CH_2Cl_2 (1:1). Evaporation of the solvents afforded **17** with high purity.
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