Development of a novel oxidatively removable and recyclable linker for combinatorial solid phase synthesis

Wen-Ren Li,* Nai-Mu Hsu, Hsueh-Hsuan Chou, Sung Tsai Lin and Yu-Sheng Lin

Department of Chemistry, National Central University, Chung-Li, Taiwan 32054, ROC.
E-mail: wenrenli@rs250.ncu.edu.tw

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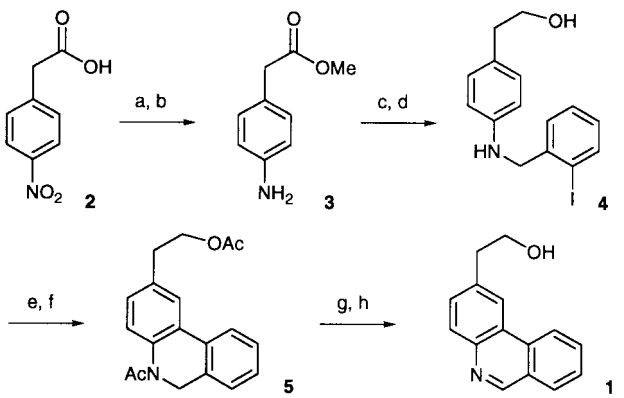
An appropriately derivatized phenanthridine is shown to behave as a novel, reusable linker which is based on a disubstituted amide anchorage and forms an acid group on oxidative cleavage, but tolerates exposure to acidic, basic and reductive reaction conditions.

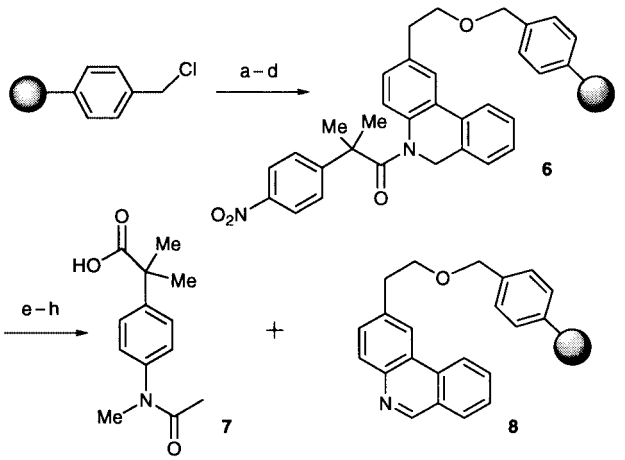
Recently, a considerable amount of attention has been focused on the use of the combinatorial library approach for the discovery of new molecules with desired properties.[1] Up to now, syntheses of a variety of classes of chemical libraries have been constructed employing solid phase methods.[2,3] Many linkers have, therefore, been developed for anchoring and selectively removing the desired target molecule from polymer supports.[4–8] As far as we are aware, none of the available linkers used for carboxylic functions survive through a series of acidic, basic and reductive reactions and are also stable to *N*-alkylation conditions.[9–11] This void led us to devise a new type of recyclable phenanthridine linker for the synthesis of acids, which possesses greater stability under the above reaction processes and the capability to be removed by a mild oxidative reagent. In addition, it can be recycled.

The synthesis of an appropriately functionalized phenanthridine **1** is detailed in Scheme 1. The synthesis was initiated with protection of the acid group of 4-nitrophenylacetic acid as its methyl ester using $SOCl_2$ and MeOH. Reduction of the nitro group of the methyl ester was rapidly accomplished by catalytic transfer hydrogenation with NH_4HCO_2 as the hydrogen donor over a Pd/C catalyst.[12] Coupling of amine **3** with 2-iodobenzoic acid was followed by reduction using $NaBH_4$ and $BF_3{\cdot}OEt_2$ to form the secondary amine **4** in 73% yield for four steps from 4-nitrophenylacetic acid. Attempts to perform an internal biaryl cyclization of the aryl substituted amide, before $NaBH_4$ reduction, gave an undesired intermolecular coupling dimer. Acylation of the amino and hydroxy group of compound **4** with AcBr, followed by intramolecular Heck type cyclization[13] employing $Pd(OAc)_2$, PPh_3 and Ag_2CO_3 in MeCN led to the amide **5** in 70% yield. Oxidative cleavage of the resulting amide **5** with cerium ammonium nitrate (CAN) and subsequent hydrolysis of the acetate with LiOH afforded the amine-masked phenanthridine handle **1** as a crystalline compound. Of particular note is that the synthesis of all intermediates in Scheme 1 can be carried out on a preparative scale to afford ample quantities of the desired compound in roughly 40% overall yield.

The suitability for reductive processing and alkylation of the new linker and its applicability to peptide chemistry were demonstrated by the synthesis a trimethylated derivative of actarit,[14] an immunomodulating agent, and a *N*-acylated dipeptide on the Merrifield resin as shown in Schemes 2 and 3. The phenanthridine derivatized solid support **8** (Scheme 2) can readily be prepared by treating chloromethylpolystyrene with the sodium salt of phenanthridine in dry DMF at room temperature for 16 h. In this form the phenanthridine resin is stable and can be stored for long periods without loss of activity. Reduction of this functionalized resin furnished the resin-bound secondary amine, which was first derivatized with 4-nitrophenylacetic acid and then subjected to enolate alkylation by employing commonly used bases such as NaH or LDA.[15] Reduction of the nitro group of resin **6** with $SnCl_2$ in DMF afforded, after acetylation and *N*-alkylation, the trimethylated acid-bound resin. Treatment of the resulting resin with 2 equiv. of CAN in MeCN and water for 10 min resulted in complete cleavage of the desired acid from the solid support. After removal of the MeCN, the crude product was dissolved in EtOAc, washed successively with water and aqueous HCl and purified to give the trimethylated actarit derivative **7** in 57% overall yield for eight steps. The regenerated phenanthridine resin **8** could be reused for another synthetic sequence with only a slight loss in activity. The yields after one and two recycles are 90 and 84%, respectively. The resin-bound amides in Scheme 2 are stable under either basic or acidic hydrolytic conditions.



Scheme 1 *Reagents and conditions*: (a) $SOCl_2$, MeOH, 97%; (b) 10% Pd/C, HCO_2NH_4, MeOH, 97%; (c) 2-iodobenzoic acid, DIC, MeCN, 84%; (d) $NaBH_4/BF_3{\cdot}OEt_2$, THF, 93%; (e) AcBr, Et_3N, MeCN, 87%; (f) PPh_3, $Pd(OAc)_2$, Ag_2CO_3, MeCN, 70%; (g) CAN, MeCN, H_2O, 95%; (h) LiOH, THF, H_2O, 95%.†



Scheme 2 *Reagents and conditions*: (a) NaH, DMF, linker **1**; (b) $NaBH_4$, $BH_3{\cdot}THF$, EtOH, 71%; (c) 4-nitrophenylacetic acid, DIC, CH_2Cl_2; (d) NaH, MeI, DMF or LDA, HMPA, THF, MeI; (e) $SnCl_2$, DMF, 88%; (f) Ac_2O, Et_3N, CH_2Cl_2; (g) NaH, MeI, DMF; (h) CAN, THF, H_2O, 92%.†

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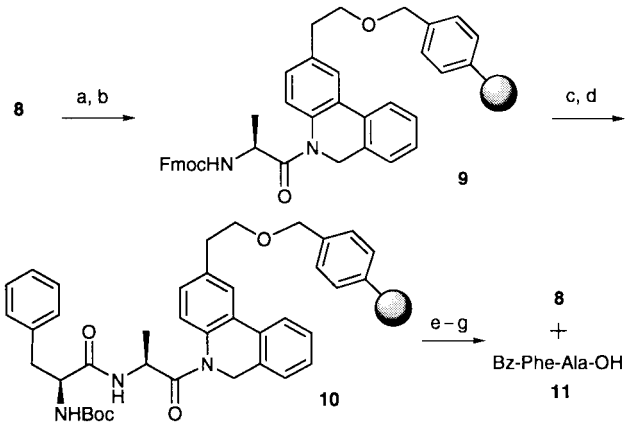

Scheme 3 *Reagents and conditions*: (a) $NaBH_4$, BH_3·THF, EtOH; (b) DIC, Fmoc-L-Ala-OH, CH_2Cl_2; (c) 20% piperidine, DMF, 89%; (d) HBTU, Pr^i_2NEt, Boc-L-Phe-OH, DMF; (e) TFA, CH_2Cl_2, 94%; (f) BzCl, Pr^i_2NEt, CH_2Cl_2; (g) CAN, THF, H_2O, 91%.

These amides were not hydrolyzed upon treatment with 1 M HCl or NaOH in THF–H_2O solution over 24 h. The further utility of this linker has been shown by synthesizing an *N*-acylated dipeptide **11** (Scheme 3). Similar results were achieved using a similar strategy to that employed for the previous syntheses. After *N*-Fmoc-L-alanine was coupled to the phenanthridine linker the resin was submitted to the peptide synthetic sequence to provide the desired dipeptide **11** in a nonoptimized yield of 76%, as shown in Scheme 3. At each step, the coupling efficiency was about 86–95% yield as monitored by photometric Fmoc determination and the ninhydrin method. Controls during these syntheses proved that the above reaction milieu did not cause either any premature cleavage or damage of the linker.

In conclusion, we have developed a new linker for solid phase organic synthesis of carboxylic acids and have successfully demonstrated its application. The advantages of this linker are: (i) it is orthogonal to the Fmoc/Boc and Boc/Bn protecting group strategies; (ii) it can be prepared in both large quantity and high purity; (iii) its attachment to the solid support is straightforward; (iv) it is anchored to the C-terminal residue by a disubstituted amide and not an ester bond, ensuring stability to *N*-alkylation and avoidance of diketopiperazine formation at the dipeptide stage; (v) the linker is rapidly cleaved under mild oxidative conditions and the reaction is very clean; (vi) the phenanthridine resin is sufficiently robust to be recovered and recycled through the reaction sequence. Therefore, the phenanthridine linker should find broad application in the field of solid phase combinatorial synthesis where orthogonal methods of release are required. Currently, we are investigating non-aqueous oxidative cleavage conditions which will afford the corresponding esters and are exploring the length and attachment position of the alkoxyacyl spacer that is needed to link the appropriate phenanthridine handle to aminated resins such as MBHA or BHA resin through an amide bond.

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Notes and references

† Abbreviations used: DIC = 1.3-diisopropylcarbodiimide, HBTU = 2-(1*H*-benzotriazol-l-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, MBHA = 4-methylbenzhydrylamine.

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