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ScienceDirect
Tetrahedron Letters 49 (2008) 2907–2910
Tetrahedron Letters

Optimized 'inverse activation' methodology for esterification of hydroxyl-functionalized resins

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Received 15 February 2008; revised 4 March 2008; accepted 5 March 2008
Available online 10 March 2008

Abstract

Since the advent of the solid-phase method for peptide synthesis, the esterification of protected amino acid derivatives onto hydroxyl-functionalized resins has been problematic on many levels. Most methods for this reaction are attended by unacceptable levels of racemization and/or dipeptide formation, or require the use of expensive reagents with difficult handling properties. Herein, we describe a straightforward, generally-applicable method for the esterification of hydroxyl-functionalized resins, in high yield and complete stereochemical integrity.

In the past several years, solid-phase peptide synthesis (SPPS) has entered a watershed period. From its early roots in basic research, SPPS has matured to a viable method for active pharmaceutical ingredient (API) manufacturing on the metric ton scale.[1,2] The large-scale synthesis of peptide drug substances, however, has placed a renewed emphasis on the synthetic details of the underlying chemistry. For instance, peptide bond formation must be performed at high and precise levels of stereochemical integrity, and chromatographic purification must be performed with both high yield and high recovery. Whereas classical synthetic methodologies sufficed to prepare research-grade peptides, commercial APIs must be manufactured to a higher degree of precision with regard to low-level impurities that could impact patient health.

A longstanding problem in peptide chemistry has been the attachment of the C-terminal amino acid to a suitably functionalized polymeric support. When the desired compound is a peptide amide, this chemistry is fairly straightforward, as mild activation chemistries can be employed under non-basic conditions. In contrast, peptide acids are more difficult to prepare, as a high level of activation is needed for the acylation of relatively unreactive hydroxyl-functionalized resins. In such situations, the reactive chemistry needed for acceptable loading efficiency is attended by a number of deleterious side reactions.[3] While several improved chemistries have been devised to address this problem,[4–7] there is still no generally applicable methodology for *clean*, *side reaction-free* esterification of hydroxyl-functionalized resins.

Three main problems are associated with the classical method for loading hydroxyl-functionalized resins.[8] First, the use of basic additives (e.g., pyridine, 4-dimethylaminopyridine, and diisopropylethylamine) during activation almost invariably gives rise to some degree of racemization via the 5(4*H*) oxazolone mechanism, resulting in diastereomeric contamination of the final product.[3] Second, most of these bases (or contaminants therein) are of a pK_b sufficient to effect Fmoc deprotection in situ, resulting in dipeptide incorporation. Third, several amino acids with reactive side-chains, notably His(Trt), Arg(Pbf), and Asn are difficult to load in high yield due to competing intramolecular side reactions.[9]

As part of an ongoing program to develop improved synthetic processes in SPPS, we aimed to develop a fundamentally different strategy for the loading of hydroxyl-functionalized resins. In this 'inverse activation' approach,

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0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.03.029

the resin-bound hydroxyl group is activated for direct S_N2 esterification by an incoming carboxylate salt. This chemistry is mechanistically superior to the classical approach (vide supra) for several reasons. First, activation of the resin-bound hydroxyl group as the electrophile obviates carboxyl activation of the C-terminal amino acid, thereby abrogating any possibility of racemization. Second, forgoing carboxyl activation of amino acid derivatives bearing reactive side-chains would result in significantly higher esterification yields due to the absence of competing intramolecular side reactions. Third, the elimination of any nucleophilic base additive during the loading step eliminates the potential for dipeptide formation by means of in situ Fmoc deprotection.

Activation of resin-bound hydroxyl groups toward esterification by incoming Fmoc amino acid carboxylate salts has been reported in recent years using a number of chemistries. The most familiar example of this chemistry is the chlorination of resin-bound trityl alcohol with chlorinating agents such as $SOCl_2$ or acetyl chloride.[10,11] Similarly, Mergler et al. reported the chlorination of resin-bound benzylic alcohols (on SASRIN® resin) using CCl_4/PPh_3 and $PPh_3{\cdot}Cl_2$ salts.[12] Activation of Wang resin as the benzylic bromide has been reported along similar lines using CBr_4/PPh_3[13] or the Me_2S-succinimide bromide salt.[14]

For a project involving the synthesis of a peptide acid, we first attempted to apply known halogenation chemistry to Wang resin for reaction with an incoming amino acid carboxylate. Chlorination of Wang resin seemed *a priori* to be ill-advised, as $SOCl_2$ and acetyl chloride themselves—as well as the resulting hydrogen chloride solution liberated—would likely cleave the acid-labile benzylic ether linkage from the solid support. Moreover, resin-bound benzylic chlorides are insufficiently reactive during the esterification reaction, requiring forcing conditions for quantitative esterification. We therefore attempted to prepare the more reactive resin-bound bromide, which could be achieved under neutral conditions, such as CBr_4/PPh_3 and $PPh_3{\cdot}Br_2$, either of which would result in a resin-bound bromide of enhanced reactivity relative to the chloride. These chemistries, however, were fraught with significant problems. In both cases, insoluble precipitate—presumably triphenylphosphine oxides or salts thereof—evolved during the activation reactions in CH_2Cl_2, THF, or toluene. These problems were unacceptable from both scaleability and loading efficiency perspectives. Even if quantitative activation were achieved (which was unlikely given the decrease in polymer accessibility as a result of precipitate formation in the resin interior), excessive washing was necessary to remove these contaminating byproducts, and verification of complete removal was difficult, if at all possible.

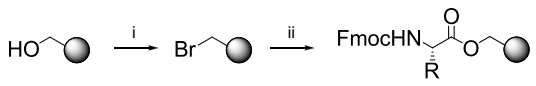
We therefore aimed to find a reagent which would result in clean bromination of resin-bound hydroxyl groups in high yield and without evolution of solid by-products. Despite its chemical similarity to $SOCl_2$ and the potential danger of linker cleavage from the resin (vide supra), we first attempted to use $SOBr_2$ as a bromination reagent since it has been reported to be compatible with Wang resin.[15] Nonetheless, $SOBr_2$ resulted in over-brominated (by elemental analysis) and purple-colored resins, presumably due to bromination of the benzylic linker moiety and/or resin backbone. We then attempted to use PBr_3 for solid-phase bromination, as this reagent has long been useful for bromination of acid-sensitive moieties and occurs cleanly in CH_2Cl_2 without evolution of insoluble byproducts. In contrast to $SOBr_2$, bromination using PBr_3 resulted in fast, clean, and near-quantitative (91%) bromination of the Wang linker under homogeneous conditions at room temperature.[16] The resulting brominated Wang resin could then be stored in dried form for later use, or immediately subjected to esterification under operationally facile conditions for all classes of commonly used Fmoc amino acids, all with excellent yields (Table 1)[17] (Scheme 1).

Several features are noteworthy from this work. First, the resin-bound benzylic bromide is of sufficient reactivity to allow for direct esterification by incoming Fmoc amino acids without an exogenous catalyst, such as CsI, or *n*-Bu_4NI. The absence of these additives allows for enhanced

Table 1
Amino acid-loaded resins prepared in this study[a]

Entry	Carboxylic acid	Loading (mmol/g)	Yield (D%)[e]	Entry	Carboxylic acid	Loading (mmol/g)	Yield (%)
1	Fmoc-Ala-OH [H_2O]	0.47	80%	11	Fmoc-Met-OH	0.49	85
2	Fmoc-Cys(Trt)-OH	0.45	88% (n.d.)	12	Fmoc-Asn-OH	0.48	83
3[b]	Fmoc-Cys(Trt)-OH	0.38	74% (10.4%)	13	Fmoc-Asn(Trt)-OH	0.45	88
4	Fmoc-Asp(O*t*Bu)-OH	0.45	80%	14	Fmoc-Pro-OH	0.47	80
5	Fmoc-Phe-OH	0.47	83%	15	Fmoc-Arg(Pbf)-OH	0.41	83
6	Fmoc-Gly-OH	0.48	80%	16	Fmoc-Ser-OH	0.45	76
7	Fmoc-His(Trt)-OH	0.41	82% (n.d.)	17	Fmoc-Ser(*t*Bu)-OH	0.46	81
8[b]	Fmoc-His(Trt)-OH	0.40	80% (49.5%)	18[c]	Fmoc-Ser(*t*Bu)-OH	0.45	71
9	Fmoc-Ile-OH	0.50	86%	19[d]	Fmoc-Ser(*t*Bu)-OH	0.43	56
10	Fmoc-Lys(Boc)-OH	0.48	88%	20	Fmoc-Trp(Boc)-OH	0.43	81

[a] Unless otherwise noted, loadings were performed on Wang resin brominated at a loading of 0.69 mmol Br/g (by elemental analysis).
[b] Loading reaction performed using DIC/DMAP activation.
[c] Loading performed on 4-methylbenzhydrol resin.
[d] Loading performed on trityl alcohol resin.
[e] Racemization during the esterification reaction, as determined by chiral RP-HPLC of isolated Fmoc amino acid after cleavage.



Scheme 1. Reagents and conditions: (i) PBr_3, CH_2Cl_2 (0.25 M), 1 h, rt; (ii) Fmoc-Xaa-OH, DIEA (2 equiv, 0.2 M), DMF, 16 h, rt.

chemoselectivity in the alkylation reaction and lower cost-of-goods. Second, the use of a resin-bound benzylic bromide as the electrophile—rather than the chloride—allows the esterification to be performed at ambient temperature. This is preferable in solid-phase chemistry for a number of reasons, notably the instability of protected amino acid derivatives at elevated temperatures and the necessity of specialized equipment for the efficient manipulation of resins at elevated temperatures. Moreover, the homogeneous bromination reaction conditions eliminate the need for extensive washings to eliminate trapped precipitate from the resin interior.

Given the success of this chemistry using Wang resin, we applied this chemistry to two different hydroxyl-functionalized resins, the 4-methylbenzhydrol (MBH) and trityl alcohol resins. MBH resin is a useful functional homologue of Wang resin, marked by comparable acid lability but with greater steric hindrance, which is of particular concern during the synthesis of diketopiperazine-prone sequences.[18,19] Trityl alcohol resin is a functional homologue of the common 2-chlorotrityl chloride resin, albeit with slightly greater acid lability and comparable benefits for diketopiperazine suppression.[20] Loading of Fmoc-Ser(*t*Bu)-OH onto MBH and trityl alcohol resins was accomplished with the same two-step sequence as with Wang resin. However, a lower yield was observed with both MBH and trityl alcohol resins (Table 1, entries 18 and 19). Given the greater reactivity of benzyl bromides relative to the more commonly used chlorinated analogs of these resins, it is possible that these lower loadings were due to either partial hydrolysis of the brominated resins by adventitious water, or steric hindrance caused by the additional phenyl rings in these linkers. Another potential causative factor is that the synthesis of these resins is fraught with harsher reagents and longer reaction sequences than for Wang resin; this can result in inaccessible loading sites in the resin interior. These explanations notwithstanding, practitioners using such resins should exercise caution and ensure that the loading level achieved is adequate for a given synthetic application.

A further benefit of using an 'inverse activation' method for loading hydroxyl-functionalized resins is its compatibility with some side-chain-unprotected amino acids. The classical regime of carboxyl activation is incompatible with such strategies due to competing intramolecular reactions. For instance, side-chain-unprotected amino acids bearing aliphatic alcohols (Ser/Thr) or primary carboxamides (Asn/Gln) cannot be activated with DIC/DMAP due to the formation of the lactone or isoimide,[21] respectively. To test the functional group tolerance of this loading chemistry, we subjected Fmoc-Ser-OH and Fmoc-Asn-OH to the esterfication conditions described herein. As expected, these weakly nucleophilic side-chains do not compete with the α-carboxylate functionality in the esterification reaction (Table 1, entries 12 and 16). This is a useful finding, as side-chain-unprotected Ser and Thr are valuable synthons for the preparation of side-chain acylated and phosphorylated peptides. Additionally, side-chain-unprotected Asn and Gln are much less expensive on a molar basis than their side-chain tritylated derivatives. It is noteworthy that these chemoselectivity benefits yield tangible results given to the residual solvents contaminating many commercially available amino acid derivatives. For instance, Fmoc-Ala-OH is commercially available only as the monohydrate; this contaminating water clearly did not present a problem in the esterification reaction (Table 1, entry 1).

As noted above, racemization is a critical concern during the attachment of protected amino acid derivatives to polymer support. In the classical esterification chemistries using DMAP or other additives, racemization usually occurs to the extent of 1–5%.[22] In the case of His(Trt) and Cys(Trt), this is usually much higher due to side-chain participation, via deprotonation of the imidazolide in the former and the inductive effect in the latter.[23,24] These two cases are therefore useful examples to demonstrate the benefits of an 'inverse activation' chemistry. Esterification of the same batch of Wang resin using classical DIC/DMAP activation gave rise to 10.4% and 49.5% racemization for Cys(Trt) and His(Trt), respectively, whereas the methodology developed herein was accompanied by *no detectable racemization* (Table 1, entries 2, 3, 7, and 8).[25]

In conclusion, the 'inverse activation' method described herein is a quantum leap over the classical method for the esterification of hydroxyl-functionalized resins for several reasons. In the cases studied, excellent esterification yields were obtained for all commonly used Fmoc amino acids. Moreover, for the two most racemization-prone Fmoc amino acids—His(Trt) and Cys(Trt)—esterification was accomplished with no detectable racemization, which is in stark contrast to the grossly unacceptable degrees of stereomutation obtained using DIC/DMAP chemistry. The excellent chemoselectivity of this method also allows for the dispensation of some side-chain protecting groups, namely aliphatic alcohols and primary carboxamides. Finally, it is noteworthy that from a practical perspective, this chemistry can be performed without specialty glassware and using inexpensive, commercially available reagents. We anticipate that this methodology will find application among investigators engaged in a variety of solid-phase synthesis activities, in both peptide and small molecule arenas.

Acknowledgments

The authors acknowledge the support of Roche Colorado for this project, and Joseph Swistok (Hoffmann-La Roche) for fruitful discussions and expert technical advice.

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