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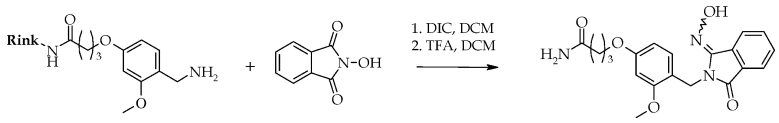

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Application of a Dual Linker with a Reference Cleavage Site to Discover a New Reaction between Amines and *N*-Hydroxyphthalimide

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Successful solid-phase organic synthesis requires careful optimization of reaction conditions for individual chemical transformations because there is no opportunity to purify polymer-supported intermediates. Typical optimization protocol involves analysis of numerous samples corresponding to different reaction conditions. The analytical technique of choice for assessing the composition of products cleaved from solid support is the LC/MS technique. To obtain meaningful data, all resin-bound components need to be released from the solid support. We have designed and used dual linkers with a reference cleavage site[1] that provide complete cleavage of all resin-bound reaction components and allow their identification using LC/MS. The concept of dual linkers is not a new one and was recently reviewed.[2] We have already reported the use of a dual linker for the identification of a side-product in the Mitsunobu transformation of polymer-supported alcohols to amines.[3] Herein, we report the use of a dual linker with a reference cleavage site in the discovery of a new reaction between amines and *N*-hydroxyphthalimide (PhtNOH).

Although not frequently used, PhtNOH was reported to be an efficient additive to suppress racemization in carbodiimide-mediated amide bond formation in peptide synthesis.[4,5] Thus, we did not expect any complication during acylation of aminomethyl polystyrene resin with 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid (HMPB linker)[6] by diisopropyl carbodiimide (DIC) in the presence of PhtNOH. After overnight reaction, no free amino groups were detected by the bromophenol blue test.[7] We continued with the reaction sequence according to Scheme 1 in an attempt to prepare a polymer-supported benzyloxyamine derivative used for the synthesis of *N*-alkyl hydroxamic acids.[8] Resin loading was evaluated by reaction of the resin-bound benzyloxyamine with *N*-(9-fluorenylmethyloxycarbonyl-oxy)succinimide (Fmoc-OSu), TFA-mediated cleavage, and UV quantification. The level of substitution was low and corresponded to only 20% of the original resin loading. The gravimetric yield of the crude product was 28%, confirming the spectrophotometrical result. The LC/MS analysis of the cleaved sample revealed the presence of the expected Fmoc-NH-OH; however, no additional product was detected. Because we had used the same reaction sequence successfully on numerous occasions[1,8,9] with *N*-hydroxybenzotriazole instead of PhtNOH, we speculated that an unexpected side-reaction had occurred during the acylation step, leading to the formation of a product not cleavable from the aminomethyl resin, which lowers the measurable yield.

To identify any resin-bound side-products, we prepared a dual linker with a reference cleavage site that allows cleavage and analysis of all products of the acylation of amino groups. A suitable dual linker was composed from Rink linker[10] as the first linker and 4-(4-aminomethyl-3-methoxyphenoxy)-butyric acid as the second linker. This dual linker, **1**, was synthesized by acylating the polymer-supported Rink linker with commercially available 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid using carbodiimide in the presence of *N*-hydroxybenzotriazole. The alcohol functionality was transformed to an amine by reaction with phthalimide under Mitsunobu conditions, followed by cleavage of the phthaloyl group with hydrazine hydrate.[11] In the dual linker **1**, the second linker is attached to the Rink linker via an amide bond representing the reference cleavage site that allows its cleavage by diluted trifluoroacetic acid (TFA).

With the resin-bound dual linker **1** in hand, we studied the reaction of its amino group with the HMPB linker in the presence of PhtNOH and DIC. LC/MS analysis of a cleaved sample revealed the presence of one major product exhibiting an ESI-MS spectrum with $m/z = 384.4$. Both the ^{1}H and ^{13}C{^{1}H} 1D NMR spectra of the HPLC purified product as well as the ESI-MS $m/z = 384.4$ signal were consistent with two isoindolinone positional isomers, **2** and **3**. To distinguish between 2-hydroxy-3-alkylimino-1-isoindolone **2** and 2-alkyl-3-hydroxyimino-1-isoindolinone **3**, the product from the reaction was analyzed in detail by both 1D and 2D ^{1}H and ^{13}C NMR spectroscopy.

Proton resonance signals were assigned to the individual hydrogen atoms on the basis of their chemical shift δ values, multiplicities, and coupling constant J values. The signals of all carbons with directly attached protons were assigned using HETCOR spectrum. Finally, the HMBC spectrum was used to assign quaternary carbons and to check the correctness of the connectivities established by the interpretation of the other spectra.

In the HMBC spectrum, the resonance signal of the H-6 proton (δ 7.94) exhibited a cross-peak with the C-1 carbonyl carbon signal at δ 161.96. Additionally, this carbonyl resonance (δ 161.96) correlated with the methylene CH_2-9 proton signal at δ 4.97. Simultaneously, both the methylene CH_2-9 proton signal and the hydroxyl proton signal (δ 11.52) showed cross-peaks with the C-2 carbon signal at δ 150.17, which indicates that the linker is attached to the endocyclic nitrogen atom, while the hydroxyl group is attached to the exocyclic nitrogen atom of the phthalimide moiety, and confirms the structure of the product as 2-alkyl-3-hydroxyimino-1-isoindolinone **3**.

With this result, indicating that neither the HMPB linker nor DIC were incorporated into the product **3**, we carried

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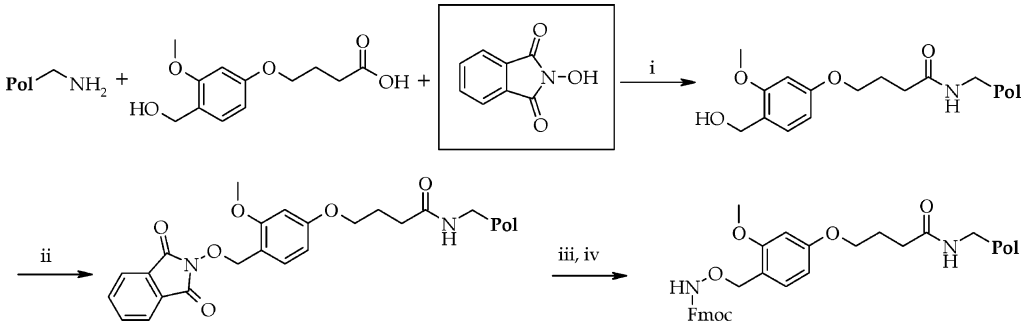
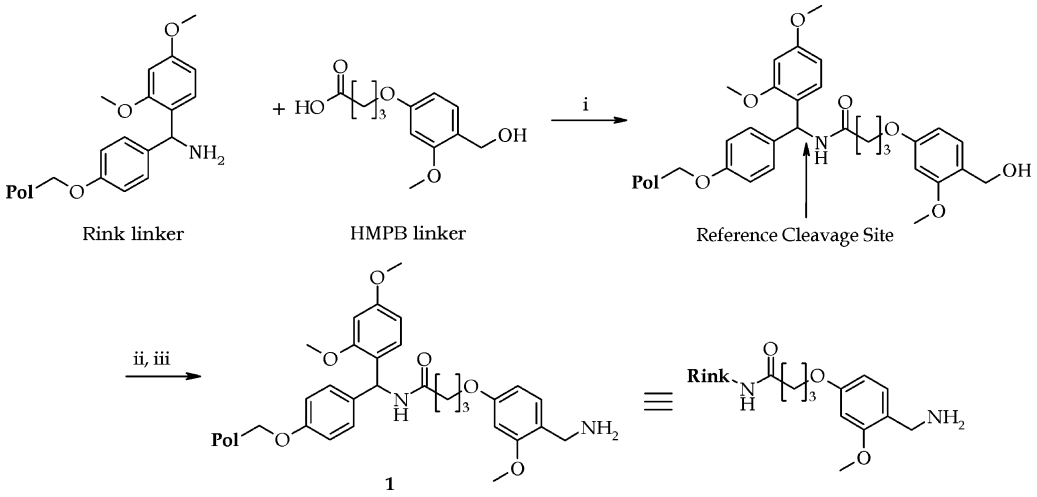
Scheme 1. Preparation of Benzyloxyamine Resin[a]



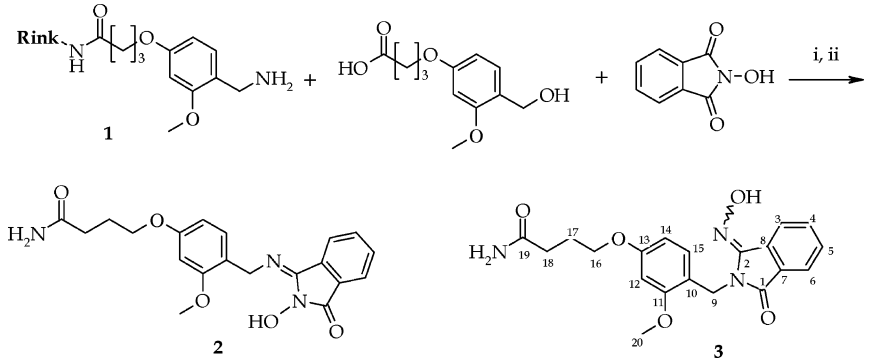
[a] Reagents: (i) DIC, DMF, 20 °C, overnight; (ii) PhtNOH, PPh_3, DIAD, anhydrous THF, 20 °C, overnight; (iii) 5% hydrazine hydrate, THF/MeOH (1:1), 20 °C, overnight; (iv) Fmoc-OSu, DCM, 20 °C, 3 h.

Scheme 2. Synthesis of the Dual Linker **1**[a]



[a] Reagents: (i) DIC, HOBt, DMF, 20 °C, overnight; (ii) phthalimide, PPh_3, DIAD, anhydrous THF, 20 °C, overnight; (iii) 5% hydrazine hydrate, THF/MeOH (1:1), 20 °C, overnight.

Scheme 3. Reaction of the Dual Linker **1** with PhtNOH in the Presence of HMPB Linker and DIC[a]



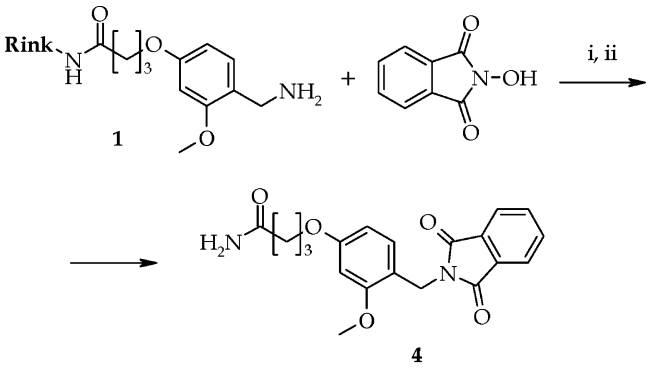
[a] Reagents: (i) DIC, DMF, 20 °C, overnight; (ii) 50% TFA in DCM, 30 min.

out the reaction of the dual linker **1** with PhtNOH in the absence of the HMPB linker and DIC (Scheme 4). The LC/MS analysis of a cleaved sample revealed the presence of one major product, which was different from isoindolinone **3**. The major product of the reaction between polymer-supported amine of the dual linker **1** and PhtNOH eluted later on a reversed-phase column when compared to 2-alkyl-3-hydroxyimino-1-isoindolinone **3** and exhibited an ESI-MS signal at $m/z = 369.3$, corresponding to *N*-alkylphthalimide **4**. The structure of the product was confirmed by the ^{1}H NMR spectrum of HPLC purified material.

The formation of *N*-alkylphthalimide **4** instead of the expected isoindolinone **3** implied that the outcome of the reaction between the polymer-supported amine and PhtNOH depends on the presence of carbodiimide. Thus, we carried out the reaction of the dual linker **1** with PhtNOH in the presence of DIC (however, in the absence of HMPB linker). Indeed, the major product of this transformation was the 2-alkyl-3-hydroxyimino-1-isoindolinone **3**, proving the decisive effect of the carbodiimide presence on the outcome of the reaction.

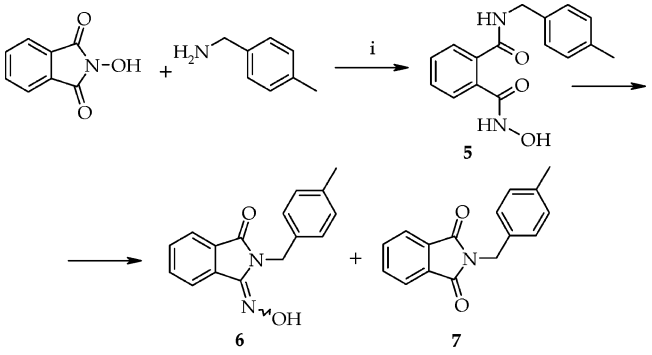
Since the formation of 2-alkyl-3-hydroxyimino-1-isoindolinones from amine and PhtNOH has not been described in the literature, we examined this chemical transformation with both components in solution (Scheme 5). Reaction of PhtNOH with 4-methylbenzylamine in DMF solution yielded

Scheme 4. Reaction of the Dual Linker **1** with PhtNOH[a]



[a] Reagents: (i) DMF, 20 °C, overnight; (ii) 50% TFA in DCM, 30 min.

Scheme 5. Reaction of PhtNOH with 4-Methylbenzylamine[a]



[a] Reagents: (i) DIC, DMF, 20 °C, overnight.

N-hydroxy-*N*′-alkylphthalamide **5** which spontaneously cyclized to *N*-alkylphthalimide **7**. An analogous reaction has been reported using *N*,*N*′-dihydroxy[2.2]paracyclophane-4,5,-12,13-tetracarboxylic acid bisimide[12] and *N*-hydroxynaphthalene-2,3-dicarboximide.[13] This route represents an alternative method for the synthesis of *N*-alkylphthalimides. Typically, *N*-alkylphthalimides are prepared by reaction of phthalic anhydride with primary amine. Since this protocol requires elevated temperature and prolonged reaction times, alternative reagents, such as *N*-(ethoxycarbonyl)phthalimide, were developed.[14]

In an attempt to prepare 2-alkyl-3-hydroxyimino-1-isoindolinone **6**, we carried out a reaction of equimolar amounts of PhtNOH, amine, and DIC. However, the product of this reaction was not the expected 2-alkyl-3-hydroxyimino-1-isoindolinone **6**, but again, *N*-alkylphthalimide **7** was detected as the major product. To explain this apparent discrepancy between these two reactions on solid phase and in solution, we studied the effect of the relative ratio of individual reaction components (Table 1). Typical solid-phase reactions are carried out with an excess of reaction components in solution and indeed, the excess of PhtNOH and DIC over the amine led predominantly to the formation of the 2-alkyl-3-hydroxyimino-1-isoindolinone **6**.

In summary, we have described a new reaction between PhtNOH and primary amines. PhtNOH reacted with primary amines, both in solution and polymer-supported, to yield *N*-alkylphthalimide. The presence of carbodiimide in an amount equimolar to PhtNOH and amine accelerated the formation of *N*-alkylphthalimide. However, when PhtNOH and DIC were in excess with respect to the amine, a typical scenario for reaction of polymer-supported amine, the outcome of the reaction was different, and 2-alkyl-3-hydroxyimino-1-isoindolinone was identified as a major product.

Table 1. Reaction of PhtNOH with 4-Methylbenzylamine in the Presence of DIC

entry	PhtNOH[a]	DIC[a]	amine[a]	t, h	**5**, %	**6**, %	**7**, %
1	0.5	0.5	0.5	24	<1	2	92
2	0.5	0.75	0.5	24	<1	4	88
3	0.5	0.5	0.75	24	<1	<1	74
4	0.75	0.5	0.5	24	23	39	20
4	0.75	0.5	0.5	48	<1	51	20
5	0.75	0.75	0.5	24	25	33	22
5	0.75	0.75	0.5	48	1	47	26
6	0.75	0.75	0.25	24	1	72	12

[a]Molar concentration in DMF.

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Supporting Information Available. Details of experimental procedures and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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