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Trimellitic anhydride linker (TAL)—highly orthogonal conversions of primary amines employed in the parallel synthesis of labeled carbohydrate derivatives☆,☆☆

Jörg Bauer and Jörg Rademann*

Institute for Organic Chemistry, University of Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany

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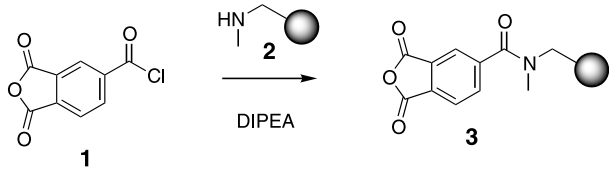
Abstract—Trimellitic Anhydride Linker (TAL) was introduced as an anchor in solid-phase synthesis allowing for immobilization of primary amines as phThALimide derivatives. 1,2-Anhydro trimellitic acid chloride (trimellitic acid = 1,2,4-benzene tricarboxylic acid) was coupled to methyl aminomethyl polystyrene at 0°C. Polymer-bound phthalimides were formed from primary amines by heating, preferably assisted by microwave irradiation, or via a condensation protocol at rt. In the latter case, the intermediary secondary amide was formed in the presence of 4-*N*,*N*-dimethylamino pyridine (DMAP), ring closure to the cyclic imide was effected smoothly in the presence of 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) as condensation agent. The linker was stable under a broad range of reaction conditions including strong acid, base, and oxidants, respectively. Efficient cleavage could be effected by treatment with 1% hydrazine or with ethylenediamine, respectively. Alternatively, products were released by a safety-catch procedure. Phthalimides were reduced with borohydrides, preferably with $LiBH_4$, and products were afforded in high purity and with excellent yields by treatment with dilute acid (5% trifluoroacetic acid (TFA)). Employment of the linker for the synthesis of labeled carbohydrates was demonstrated involving efficient solid-supported glycosylation.

Organic syntheses on solid phase[1] rely on anchoring groups cleaved under specific reaction conditions which have to be compatible with the target molecules but which have to be orthogonal to the entire reaction sequence conducted on solid support.

In many instances these anchoring groups—usually denominated as linkers[2]—are derived from protecting groups thoroughly investigated in solution phase chemistry before.[3] To date several methods are available for the immobilization and release of primary amines. Predominantly, acidic conditions are employed for release of the amine products as this is the case with the various trityl resins.[4] Resins based on the 4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde) protecting group (Dde-resins) have been introduced for basic release of amines,[5] however, they are easily transamidated by primary amines and were not useful for our synthetic goals. For many purposes amine immobilization is required abolishing the nucleophilicity and basicity of the nitrogen functionality and being stable under acidic, basic, and oxidative reaction conditions. In addition, our synthetic objectives in the area of carbohydrate chemistry required amine protection being compatible with harsh (Lewis) acidic conditions as e.g. in glycosylation or aldol reactions. For these demands currently no appropriate amine linker was available.

Particularly in carbohydrate chemistry phthalimide protection of amino sugars has been appreciated in the synthesis of complex oligosaccharides.[7] Versatile reac-



Scheme 1. TAL-linker for the immobilization of primary amines.

Keywords: solid-phase synthesis; combinatorial chemistry; carbohydrates; amines.

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* Corresponding author. Tel.: +49(0)70712976197; fax: +49(0) 7071295560; Internet: http://www.uni-tuebingen.de/rademann; e-mail: joerg.rademann@uni-tuebingen.de

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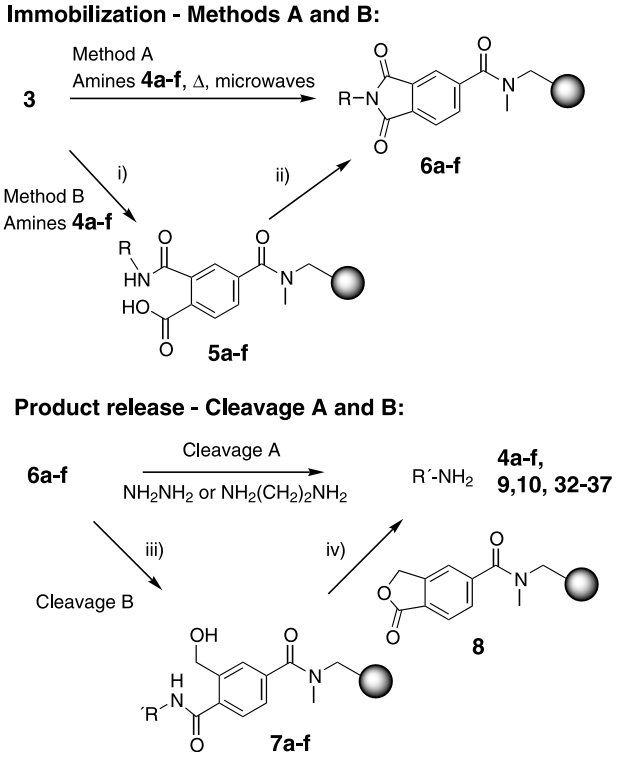
tion conditions ranging from glycosylations conducted at strongly acidic conditions, to the basic conditions required for trichloroacetimidate preparation and for the cleavage of acyl protection groups have been tolerated by the phthalimide protection group. If facilitated removal of cyclic imides was demanded, phthalimides substituted with electron-withdrawing groups[8] or non-aromatic cyclic imides[9] were employed. On solid support, phthalimide have been employed in the Gabriel reaction,[6] supported aliphatic cyclic imides have been assessed for use in oligonucleotide chemistry.[10]

The immobilization of primary amines on supported phthalic anhydrides as imides, followed by transformations and release, has not been demonstrated yet. We anticipated that trimellitic acid (1,2,4-benzenetricarboxylic acid) should be an ideal precursor for solid-supported phthalyl derivatives. The attachment of the phthalyl group via a carboxamide junction should be a favorable solution for our purposes. The electron-withdrawing effect of the carboxy-group will facilitate product release from the resin. Second by this approach we are able to avoid the potentially acid-labile and thus troublesome benzyl ether linkage.

1. Preparation of TAL-resin 3

Commercially available 1,2-anhydrotrimellitic acid chloride **1** was selected as starting material. Coupling to *N*-methyl aminomethyl polystyrene resin (1.36 mmol g^{-1}) in the presence of diisopropylethyl amine (DIPEA) as base resulted in the formation of the 4-carboxamide **3** with 1.10 mmol g^{-1} (Scheme 1). No competing attack of the cyclic anhydride was observed. The intact phthalic anhydride displayed two characteristic signals in the FT-ATR IR spectrum at 1854 and 1782 cm^{-1} (Fig. 1) and was stable during storage at rt.



Scheme 2. Immobilization of primary amines on TAL-resin **3**. Method A: MW, 200°C, 5 min. Method B: (i) 3 equiv. **4a–f**, DMAP, DMF, rt; (ii) MSNT, NMI, DCM. Product release: Cleavage A: NH_2NH_2, THF, 65°C. Cleavage B: (iii) $LiBH_4$, THF, 90 min; (iv) 5% TFA, 5% water, DCM, 2 h.

2. Amine immobilization

Loading of TAL resin **3** with various primary amines (**4a–f**) proceeded via the secondary amide **5a–f** to the cyclic imides **6a–f** (Scheme 2, top). The formation of **6** could be effected by thermal immobilization (Method A). For aliphatic or benzylic amines reaction to the phthalimides (**6a–f**) was completed in 4 h at 90°C in DMF. Anilines required significantly elevated temperatures (110°C, argon atmosphere). Thermal loading was greatly facilitated by using a microwave synthesizer. Quantitative loading of resin **3** was effected in 5 min with 3 equiv. of **4a–f** in DMF at 200°C in a closed reaction vessel.[11] To avoid the harsh conditions of thermal loading, we focussed our efforts on developing a novel and smooth protocol leading to complete loading of resin **3** at room temperature (Scheme 2, top) (Method B). Ring-opening of **3** to the secondary amides **5a–f** proceeded smoothly already at rt in the presence of *N*,*N*-dimethylamino pyridine (DMAP) as acylation catalyst. Consequently, the ring closure of **5** was investigated with acids and various condensing agents. The superior method for the smooth formation of imides **6a–f** was the treatment with 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) and *N*-methylimidazole (NMI) in DCM at rt.[12] Formation of resin **6** could be followed by FT-ATR-IR (Fig. 1).

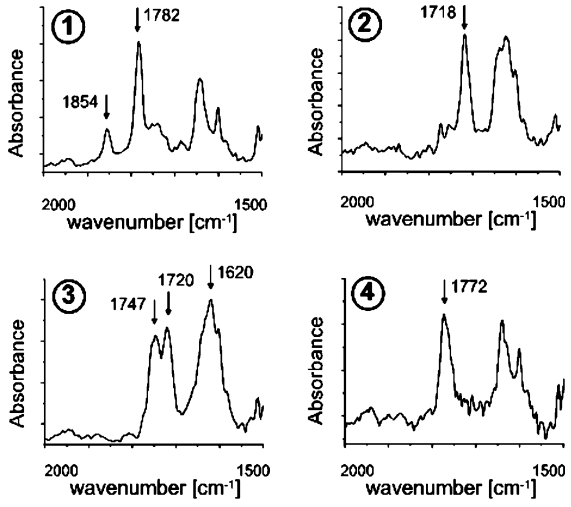


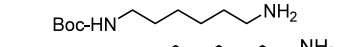
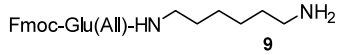
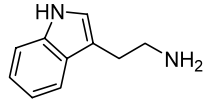
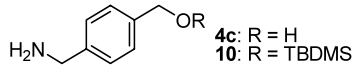
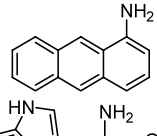
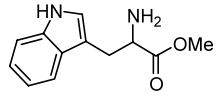
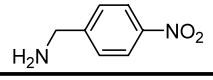
Figure 1. FT-ATR-IR monitoring of a synthetic sequence on the TAL resin: (1) Anhydride resin **3**. (2) Phthalimide resin **6**. (3) Glycosylated resin **27**. (4) Benzolactone resin **8** after product release.

The anhydride bands disappeared and the phthalimide was detected at 1712–1720 cm^{-1}. The loading was quantified by product cleavage and gravimetric yield determination (Table 1). In addition, the resin loading was measured by Fmoc release. Boc-protected diamine **6a** was deprotected (3×15 min 20% TFA in DCM) and coupled with Fmoc-Glu(All)-OH activated with TBTU[13] and DIPEA (3 equiv. each). Fmoc was released and quantified spectrophotometrically indicating a loading with amine **9** (see Table 1) corresponding to a 98% conversion of **3**.

3. Conversions and linker stability

Various conversions of immobilized amines **6** were investigated. Boc-deprotection, TBTU-coupling, silylation, and glycosylation reaction proceeded smoothly to completion (Table 1) Stability of the polymer-supported phthalimide was investigated with resin **6b** as a model compound. Stability was evaluated by the intact imide band in single bead FT-ATR-IR and by product release as analyzed by HPLC. The linker was stable to 5 equiv. of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in DCM and towards 95% TFA. It was not cleaved by secondary and tertiary amines and by 0.1 M 1,8-diazabicyclo[5.4.0]undec-7-en (DBU). By dilute sodium methoxide in MeOH/DCM (15 μL of 0.5 M NaOMe/MeOH in 5 mL DCM, pH 9 on wet pH paper) the linker was not affected. However, we observed cleavage of the phthalimide **6b** with a 0.5 M solution of NaOMe in DCM/MeOH 2:1. Thus, the TAL-resin is offering an attractive stability profile for carbohydrate chemistry including Lewis-acidic and basic deacylation conditions.

Table 1. Yields and HPLC purities (214 nm) of amines released from resin **6a**–**f** by nucleophilic cleavage A and reductive cleavage B. [+] Product mixture of **4e** and the respective hydrazide

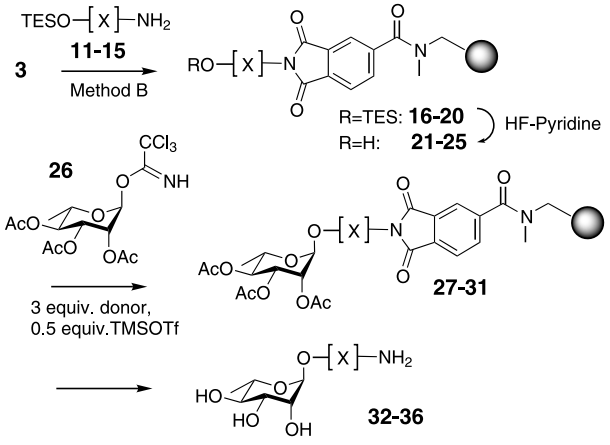
Amines	Released Product	A	B
4a		88 (92)	63
4a	 **9**	--	82 (97)
4b		86 (99)	99 (96)
4c	 **4c**: R = H **10**: R = TBDMS	88 (90) 87 (99)	90 (99) --
4d		68 (99)	76 (89)
4e		$(66+29)^{+}$	(90)
4f		96 (95)	84 (95)

4. Product release

Depending on the stability of the target molecule different cleavage conditions are desirable. Thus, we have developed two complementary cleavage protocols. Acid-sensitive products were efficiently released by treatment with 1% hydrazine hydrate in THF (4 equiv.) at 65°C (Scheme 2, bottom) (Cleavage A). Following the evaporation of solvents, *tert*-butanol/ water was employed for subsequent lyophilization. Hydrazine could be substituted by ethylene diamine (8 equiv.) with identical results. In both cases the complete removal of the base was easily achieved as judged by mass spectrometry. For the release of base-sensitive product molecules a convenient safety-catch protocol (Scheme 2, bottom) (Cleavage B) was superior to nucleophilic release. The solid-supported phthalimides **6a**–**f** were reduced by $LiBH_4$ in THF (90 min, rt) yielding the secondary amides **7a**–**f**. The reaction was accompanied by the disappearance of the 1718 cm^{-1} band in the IR spectrum. Reduction with $NaBH_4$ in THF/MeOH proceeded significantly slower requiring 6 h to be completed.[14] Primary amines were released upon treatment with dilute acid (5% TFA, 5% H_2O in DCM). The primary alcohols **7a**–**f** cyclized to benzolactone **8** which could be observed in the IR-spectrum at 1772 cm^{-1}. The efficiency of cleavage procedures A and B was investigated in respect to a collection of polymer-supported phthalimides, product purities were determined by HPLC (214 nm, Table 1). Yields determined by weight were between 80 and 95% both for cleavage procedures A and B.

5. Synthesis of labeled carbohydrates on the TAL-resin

Several linkers have been devised for the polymer-supported synthesis of carbohydrates.[15] To demonstrate the feasibility of TAL-resin **3** for the synthesis of carbo-



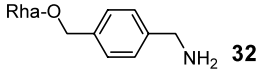
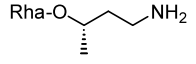
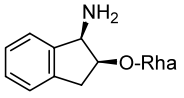
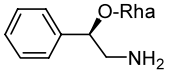
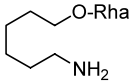
Scheme 3. TAL-resin for the immobilization and release of carbohydrate derivatives.

hydrate-conjugates we prepared a small collection of amine-labeled sugars (Scheme 3). Carbohydrates carrying diverse amine labels are important starting compounds for carbohydrate ligation. Thus, five triethylsilyl (TES)-protected aminoalcohols were coupled to resin **3** employing the two step immobilization method B (first amine/DMAP, then MSNT). Following smooth removal of the TES-groups employing HF-pyridine, the trichloroacetimidate of L-rhamnose **26**[16] (3 equiv.) was activated in DCM at 0°C with 0.5 equiv. of TMSOTf in the presence of molecular sieves. The resin-bound glycosides **27**–**31** were analyzed by single-bead FT-ATR-IR (see Fig. 1, 3) and by suspension ^{13}C NMR. Complete glycosylation of **6c** was proven by cleavage, no amine **4c** was detected in the HPLC chromatogram. With hydrazine (Cleavage A) the fully deacetylated and labeled α-L-rhamnosides **32**–**36** were cleaved, isolated by chromatography and were characterized by ^{1}H and ^{13}C NMR, and by high-resolution MS (Table 2). Isolated yields are acceptable to good for multi-step micro-scale preparations. The obtained products are useful starting materials for the preparation of diverse glycoconjugates. Currently, we employ the described methodology to the preparation of immune-stimulating glycoproducts.

6. Summary

The TAL-resin offers an attractive alternative to previous methods for amine immobilization and release. The stability range of the novel support is exceptionally wide and might allow novel applications that did not succeed on the current support materials. Reported limitations of the phthalyl group have been overcome by introducing novel procedures for amine immobilization and product release at ambient temperature or with microwave irradiation. The versatility of the novel resin has been proven by immobilization and cleavage of chemically diverse amines. The parallel preparation of amine-labeled carbohydrates via solid-supported glycosylation reactions was demonstrated successfully and will be employed in the synthesis of bioactive glycoconjugates.

Table 2. Synthesis of labeled carbohydrates **32**–**36**

Aminoalcohol TESO-[X]-NH_2	Product[a]	Crude [mg]	Yield [mg] [(%)]
11	 **32**	8.8	6.2 (54)
12	 **33**	10.1	5.9 (56)
13	 **34**	13.8	5.6 (35)
14	 **35**	14.2	8 (54)
15	 **36**	11.3	7.4 (49)

[a] Rha = α-L-Rhamnopyranosyl-

7. Supporting information available

Detailed descriptions of the experimental procedures and fully assigned ^{1}H and ^{13}C NMR data of compounds **32**–**36** are provided in the Supporting Information (5 pages).

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