Synthesis S. Bhilare et al. PSP

Scale-Up of a Heck Alkenylation Reaction: Application to the Synthesis of an Amino-Modifier Nucleoside 'Ruth Linker'

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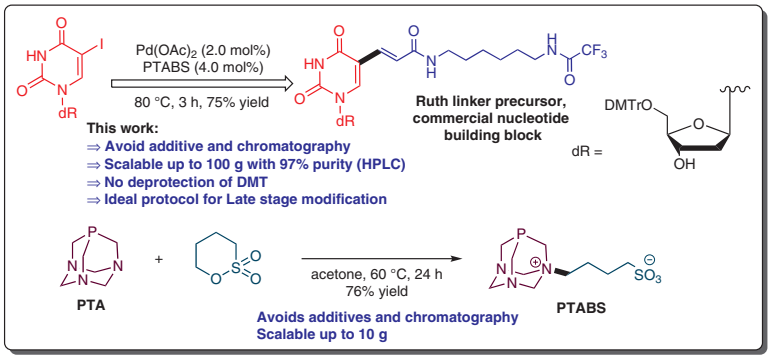

Dedicated to the memory of Jerry Lynn Ruth who first demonstrated the utility of this molecule for DNA labeling

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Abstract Ruth linker is a C5 pyrimidine modified nucleoside analogue widely utilized for the incorporation of a primary amine in a synthetic oligonucleotide. The increasing demand for non-radioactive labeling, detection of biomolecules, and assembly of COVID-19 test kits has triggered a need for scale-up of Ruth linker. Herein, an efficient protocol involving a palladium-catalyzed Heck alkenylation is described. The synthesis has been optimized with a goal of low catalyst concentration, column-free isolation, high product purity, reproducibility, and shorter reaction time. The scalability and utility of the process have been demonstrated successfully on a 100 g scale (starting material). Additionally, for scale-up of the Heck alkenylation protocol, 7-phospha-1,3,5-triaza-adamantanebutane sulfonate (PTABS) as the coordinating caged phosphine ligand was also synthesized on a multigram scale after careful optimization of the conditions.

Key words nucleosides, Heck reaction, alkenes, palladium, catalysis, cross-coupling, homogeneous catalysis

1 Introduction

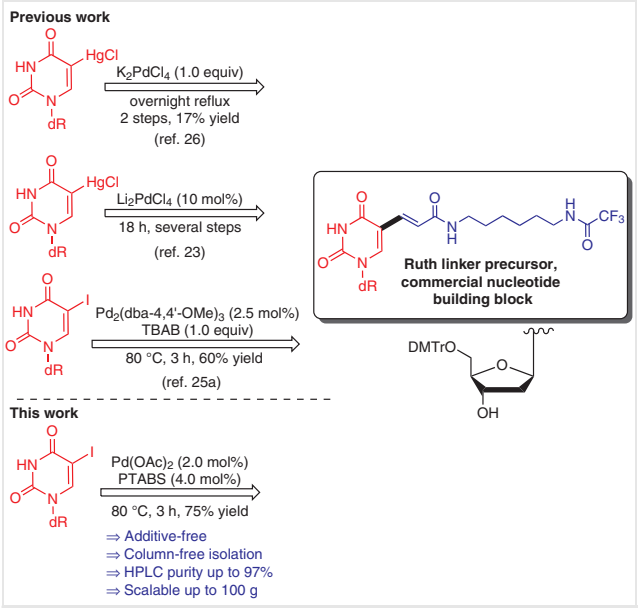
Nucleic acids are biomolecules with special significance as they are directly involved in the various cellular functions like replication, transmission, and transcription of genetic information. Nucleosides are structural subunits of nucleic acids and building-blocks of natural DNA or RNA consisting of a heterocyclic aglycone part (purine and pyrimidine bases) essential for Watson–Crick base pairing.[1,2] The structural propensity of the nucleoside substructure to undergo chemical modification has been exploited by researchers in recent decades for the development of biological/fluorescent probes for DNA imaging applications as well as nucleoside-based pharmaceutical drugs for the treatment of cancer and viral infections.[3–5] As a testament to the rapid growth of this field of research, more than 40 nucleoside analogues have in recent years been approved as drug candidates while several are in clinical trials.[6,7] Majority of the structural modifications that have been undertaken on the nucleoside substructure was achieved by the functionalization of the heterocyclic bases.[8]

Modification of nucleobase under mild condition can be efficiently accomplished by the employment of transition-metal-catalyzed cross-coupling reactions that are well known for providing excellent stereo-, regio- and chemocontrol.[9] The success of transition-metal-catalyzed processes, in general, has been attributed to the rapid development of a wide variety of catalytic systems that have paved the way for construction of C–C and C–heteroatom (such as C–N, C–O, and C–S) bonds that are either difficult or time-consuming via the conventional synthetic methods.[10,11] Transition-metal-catalyzed processes have over the years also achieved higher degrees of sustainability, increased productivity, practical industrial applicability and with the ease of incorporation of green chemistry aspects such as recyclability, minimization of waste, and scalability.[12] Amongst the variety of transition-metal-catalyzed processes, palladium-based cross-coupling reactions find a special

place and have received widespread utility not only in academia but in numerous industrial processes for drug discovery and agrochemical applications.[13]

Palladium-catalyzed processes have also been employed for the modification of nucleosides reported by Ruth and Bergstrom in 1976 in the form of Heck alkenylation of uridine at the C-5 position.[14–16] Since these early reports, application of palladium-catalyzed cross-coupling, especially Heck alkenylation reactions has come a long way in efficiently modifying the purine as well as the pyrimidine bases as per the required applications either as functional probes or drugs.[17] Brivudine or BVDU (antiviral drug) is an excellent example to highlight the synthetic prowess of palladium-catalyzed Heck alkenylation for commercial applications.[1,18]

Another C-5 alkenylated nucleoside of 2′-deoxyuridine that has found wide commercial relevance is Ruth linker allowing the incorporation of a primary amine with a 10-atom spacer anchored on a pyrimidine base within an oligonucleotide.[19–23] The long spacer is designed to project the arm into major groove of the double-stranded DNA without impeding on the hybridization (Scheme 1). The easy accessibility and reactive site make Ruth linker ideal for conjugation of reporter groups and fluorescent dyes offering both enhanced sensitivity and signal amplification for the detection and quantification of pathogens including COVID-19.[24,25]



Scheme 1 Schematic summary for the synthesis of Ruth linker

As demonstrated in 2002 by Walton et al., the Ruth linker was employed as a reagent for the synthesis of internally labeled DNA.[26,27] Therefore, the ability to hybridize complementary nucleic acid probes for the detection of a specific target sequence has revolutionized the DNA-based diagnostic industry. Particularly, non-radioactive hybridization probes offer the speed, sensitivity, safety, and ease of analytics.[15,27]

The original synthetic route reported by Ruth and subsequently improved by Walton et al. utilized a palladium-catalyzed Heck alkenylation reaction using an organomercury derivatized nucleoside with an alkene coupling partner. Besides using stoichiometric amounts of the palladium catalyst (K_2PdCl_4) and a toxic organomercury compound, the protocols also suffered from poor reactivity (Figure 1). Another important aspect that needs to be considered towards the scalability of such a protocol would be the isolation of the product without column chromatography. From the point-of-view of the post-synthetic applicability of the synthesized nucleoside derivative, 5′-*O*-DMTr protection needs to be carried out and further losses would limit the overall product yield.[28,29]

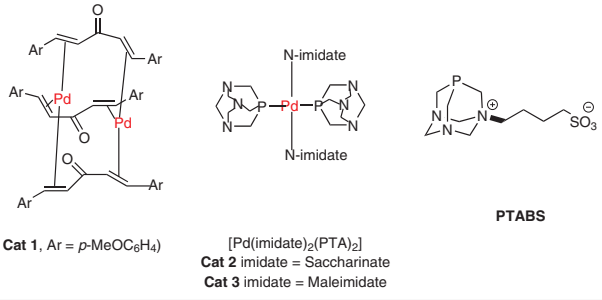


Figure 1 Catalytic systems used for cross-coupling reactions

These shortcomings to a certain extent were recently addressed by our research group with the employment of [Pd_2(dba-4,4′-OMe)$_3$] (**Cat 1**, Figure 1) as a precatalyst for catalyzing Heck alkenylation of 5′-*O*-DMTr-5-iodo-2′-deoxyuridine with alkene at a catalyst loading of 2.5 mol% (5.0 mol% Pd concentration). An improvement in the yield of the DMTr-protected cross-coupled product was realized, however, the catalyst concentration, as well as the yield, remained a concern.

Our research group has over the years developed more efficient palladium-based catalytic systems involving caged phosphine ligands such as triazaphosphaadamantane (PTA) and its derivatives (PTABS and PTAPS).[30] These ligand systems either as complexes of palladium (e.g., PTA complexes such as [Pd(Sacc)$_2$(PTA)$_2$] **Cat 2** or [Pd(Mal)$_2$(PTA)$_2$] **Cat 3** shown in Figure 1)[31–33] or in situ activation with a palladium precursor [PTABS with $Pd(OAc)_2$][34] have been effective in catalyzing the modification of nucleosides (Suzuki–Miyaura, Heck alkenylation, Sonogashira coupling, aminocarbonylation)[30,35] as well as the functionalization of chloroheteroarenes (amination, etherification, and thioetherification).[36–38] In 2015, utilization of [Pd(Sacc)$_2$(PTA)$_2$] catalytic system in catalyzing the Heck alkenylation at 1.0 mol% concentration for 5′-*O*-DMTr-5-iodo-2′-deoxyuridine failed

to obtain the desired product in good yield, although coupling with the unprotected 5-iodo-2′-deoxyuridine was successful.[26]

We, therefore, switched our attention to Pd/PTABS catalytic system that has proven to be the most efficient for numerous cross-coupling reactions. In this report, we would like to document our findings of developing a single-step scale-up (100 g) of Heck alkenylation protocol for the synthesis of Ruth linker from 5′-*O*-DMTr-5-iodo-2′-deoxyuridine. To achieve this goal, the synthesis of phosphine ligand PTABS was also necessary and carried out on a multi-gram scale by careful screening of the conditions for achieving higher yield and product purity. Other salient features of the process developed for Heck alkenylation protocol include reduction in catalyst loading, column-free isolation, additive-free synthesis, mild reaction conditions compatible towards acid-labile DMTr as well as base-labile TFA protecting groups.[25,39–44]

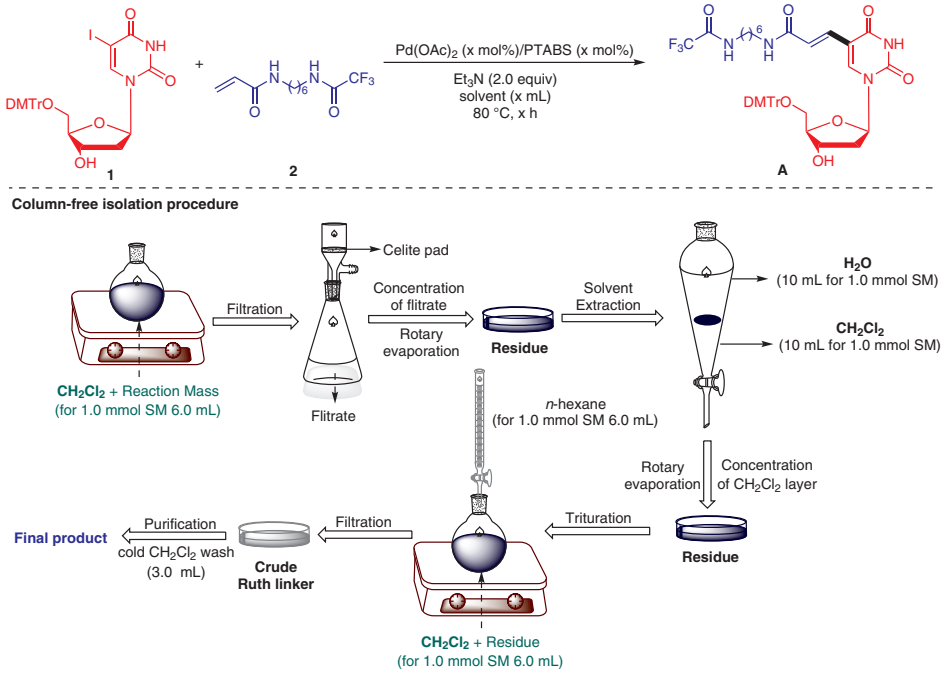
2 Results and Discussion

Literature protocols for the synthesis of Ruth linker suffer from several practical problems making the scale-up difficult and less efficient. Herein, we embarked on the development of a sustainable protocol encapsulating the following attributes: (i) column-free isolation; (ii) mild reaction parameters where both an acid-labile DMTr and base-labile N-TFA group would survive; (iii) reduced concentration of Pd catalyst; (iv) avoiding the addition of additives for catalysis; (v) improved reactivity and reduced reaction time; and (vi) scalable to 100 g with the highest purity possible for the amidite synthesis.[29]

2.1 Process Optimization

At the outset of our studies, Heck alkenylation of 5′-*O*-DMTr-5-iodo-2′-deoxyuridine (**1**) with acrylamide linker **2** using $Pd(OAc)_2$/PTABS catalytic system was performed under different catalytic conditions, varying solvents, catalyst concentration and reaction time (Scheme 2 and Table 1). It was decided at the start of the optimization studies not to employ any additive such as TBAB or other quaternary ammonium salts (commonly added in Heck reaction for the stabilization of possible Pd colloids/nanoparticles). We initiated the optimization studies by conducting the first two experiments in DMF (6 mL) and MeCN (6 mL) as the reaction solvents, respectively, at 1 mol% $Pd(OAc)_2$ concentration and 2 mol% PTABS concentration at 80 °C for 24 hours (Table 1, entries 1, 2). Product formation in both the reactions was analyzed using HPLC system (TLC analysis also performed but a more quantitative analysis technique was necessary) to suggest that it did not proceed to completion even after 24 hours (DMF 90% conversion, entry 1; MeCN 95% conversion, entry 2).

These initial results are indicative of the fact that the catalyst concentration [1.0 mol% $Pd(OAc)_2$/2.0 mol% PTABS] might not be sufficient as it failed to provide complete conversion, while the reaction time also was longer. Next, MeCN was chosen as the preferred solvent, first for providing



Scheme 2 Process optimization studies for Ruth linker synthesis using Pd/PTABS catalytic system

Table 1 Process Optimization Parameters[a]

Entry	5'-*O*-DMTr-5-IdU (mmol)	Alkene (mmol)	$Pd(OAc)_2$ (mol%)	PTABS (mol%)	Et_3N (mmol)	Solvent (mL)	Time (h)	% Conversion[b] (isolated yield, %)[c]
1[a]	1.0	1.1	1.0	2.0	2.0	DMF (6)	24	90
2[a]	1.0	1.1	1.0	2.0	2.0	MeCN (6)	24	95
3[a]	1.0	1.1	2.0	4.0	2.0	MeCN (6)	3	100 (75)
4[a]	1.0	1.1	3.0	6.0	2.0	MeCN (6)	2	100 (76)
5[d]	1.0	1.1	2.0	4.0	2.0	MeCN (6)	3	100 (72)
6[e]	1.0	1.1	2.0	4.0	2.0	MeCN (6)	3	100 (74)
7[f]	1.0	1.1	2.0	4.0	2.0	MeCN (6)	3	100 (72)
8[a,g]	5.0	5.5	2.0	4.0	10.0	MeCN (30)	2	100 (72)
9[a,h]	5.0	5.5	3.0	6.0	10.0	MeCN (30)	3	100 (72)
10[i,j]	5.0	5.5	2.0	4.0	10.0	MeCN (30)	3	100 (72)

[a] Unless otherwise mentioned, reaction conditions are 1.0 mmol of **1**, 1.1. mmol of **2**, Analar (AR) grade MeCN, AR grade Et_3N under N_2 atmosphere.
[b] HPLC conversion.
[c] Isolated column-free yield.
[d] Commercial grade MeCN used instead of AR grade MeCN while all other parameters are the same as footnote a.
[e] Commercial grade Et_3N used instead of AR grade Et_3N while all other parameters the same as footnote a.
[f] H_2O (2% weight to solvent volume) was added while all other parameters the same as footnote a.
[g] HPLC purity of >97%.
[h] HPLC purity of >92%.
[i] Commercial grade MeCN used instead of AR grade MeCN and commercial grade Et_3N used instead of AR grade Et_3N, keeping all other parameters the same as footnote a.
[j] HPLC purity of >95%.

slightly better conversion than DMF and second, the easy removal of MeCN (lower boiling point than DMF) facilitated the purification process. Subsequently, the catalyst concentration was increased to 2.0 mol% $Pd(OAc)_2$/4.0 mol% PTABS. Increased catalyst concentration was found to have a pronounced effect on the speed of the reaction where the complete conversion was accomplished in 3 hours.

To develop a column-free isolation protocol, the reaction mass obtained after completion of the reaction was diluted with CH_2Cl_2 (6 mL for 1.0 mmol SM) and stirred for 10 minutes at ambient temperature. This solution was filtered through Celite for the removal of solids and the filtrate concentrated on a rotary evaporator. The residue was then transferred to a separating funnel and was partitioned between water (10 mL for 1.0 mmol SM) and CH_2Cl_2 (10 mL for 1.0 mmol SM) and the organic layer concentrated. The aqueous washing was helpful in the removal of all the inorganic salts as well as Pd/PTABS complex. PTABS is a highly water soluble (506 mg/mL) zwitterionic caged phosphine ligand, which upon coordination to Pd metal will make the resultant complex soluble in the aqueous phase. The concentration of the organic phase provided a residue, which was further subjected to precipitation. Various solvent systems were tested for precipitation of the Ruth linker at ambient temperature and a combination of 1:1 (v/v) CH_2Cl_2 and *n*-hexane was found to be ideal.

For example, dropwise addition of *n*-hexane into a CH_2Cl_2 solution of **A** while stirring at room temperature furnished a white precipitate. Upon filtration and washing the solids with cold (0 °C) CH_2Cl_2 furnished Ruth linker as free-flowing off-white solid (Table 1, entry 3). Further improvement in the yield was possible when 3.0 mol% $Pd(OAc)_2$/6.0 mol% PTABS concentration was utilized (entry 4, 76%) while the reaction time was also reduced to 2.0 hours. However, for scale-up, benefit of increasing the concentration of Pd catalyst is not significant despite of the fact that the reaction time was somewhat shorter. First set of reactions were performed using AR grade solvents and reagents under N_2 atmosphere often used for Heck reaction.

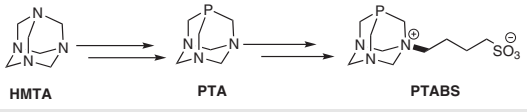
In order to reduce the cost-of-goods, next set of experiments were performed to assess the compatibility of the Pd/PTABS in commercial grade solvent/reagents instead of AR grade. First, replacement of AR grade MeCN with commercial grade MeCN allowed quantitative conversion without compromising the isolated yield of the desired product suggested no impact on the outcome of the catalytic reaction (Table 1, entry 5, 72%). Similar results were obtained by replacing AR grade Et_3N with commercial grade Et_3N (entry 6, 74%). To understand the impact and tolerance of water in commercial grade solvents, 2% volume of water was intentionally added during reaction (entry 7). Gratifyingly, no change in the catalytic activity was observed confirming the compatibility of Pd/PTABS in the presence of trace amount of water in commercial grade Et_3N and MeCN.

During the process optimization study, we also screened the exotherm generated during the reaction, which is also an important parameter during the scale-up process and was explained recently by Yang et al.[45] We did not observe any temperature excursion during the reaction.

Next, a scale-up of 5.0 mmol was undertaken to test the robustness and reproducibility of the developed protocol. The isolation of 72% Ruth linker with HPLC purity of 97% reaffirmed the reproducibility of the new process (Table 1, entry 8). The same reaction at higher catalyst concentration [3.0 mol% $Pd(OAc)_2$/6.0 mol% PTABS] was also performed to assess whether any further improvement in yield/reaction time could be achieved (entry 9). Upon isolation of the desired product (72%) and comparison with entry 8 indicated small improvement in yield but lower HPLC purity (92%). Furthermore, a 5.0 mmol reaction was conducted with commercial-grade reagents (MeCN and Et_3N) to ensure consistency (entry 10). Comparable yields (72%) and high HPLC purity (>95%) confirmed the reproducibility of the new and improved chromatography free protocol. To perform a scale-up reaction on 100 g, it was essential to synthesize the caged phosphine ligand, PTABS on multigram quantity. The following section describes the optimization and scale-up of PTABS.

2.2 Optimization and Scale-Up of PTABS

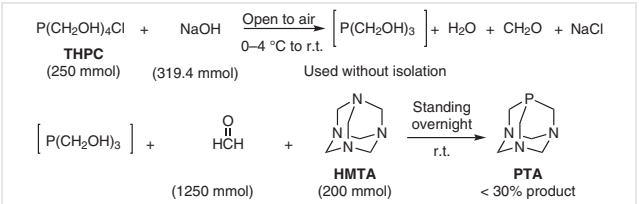
The usefulness of PTABS as caged phosphine ligand in palladium-catalyzed cross-coupling reactions was first demonstrated by our group for a variety of applications.[32–35] Small-scale synthesis of zwitterionic ligand was accomplished by the reaction of 1,3,5-triaza-7-phosphaadamantane (PTA) with 1,4-butanesultone (Scheme 3).[34] Therefore to achieve the large-scale synthesis of PTABS, PTA would be required on a multigram scale.



Scheme 3 Synthetic route for PTABS scale-up

The synthesis of PTA has been reported on a multigram scale by Daigle starting from a commercially available cheap starting material, hexamethylenetetramine (HMTA).[46,47] This procedure was further revised by other researchers.[41,48] The Daigle procedure involved the conversion of tetrakis(hydroxymethy1)phosphonium chloride (THPC) to tris(hydroxymethy1)phosphine, which then reacted in the presence of formaldehyde and hexamethylenetetramine to provide PTA (Scheme 4). The protocol was repeated with THPC (250 mmol) and NaOH (319.4 mmol) by stirring in an open-to-air beaker at 0–4 °C. The reaction mixture was slowly allowed to warm to room temperature resulting in the formation of tris(hydroxymethyl)phosphine that was used without isolation in the next reaction.

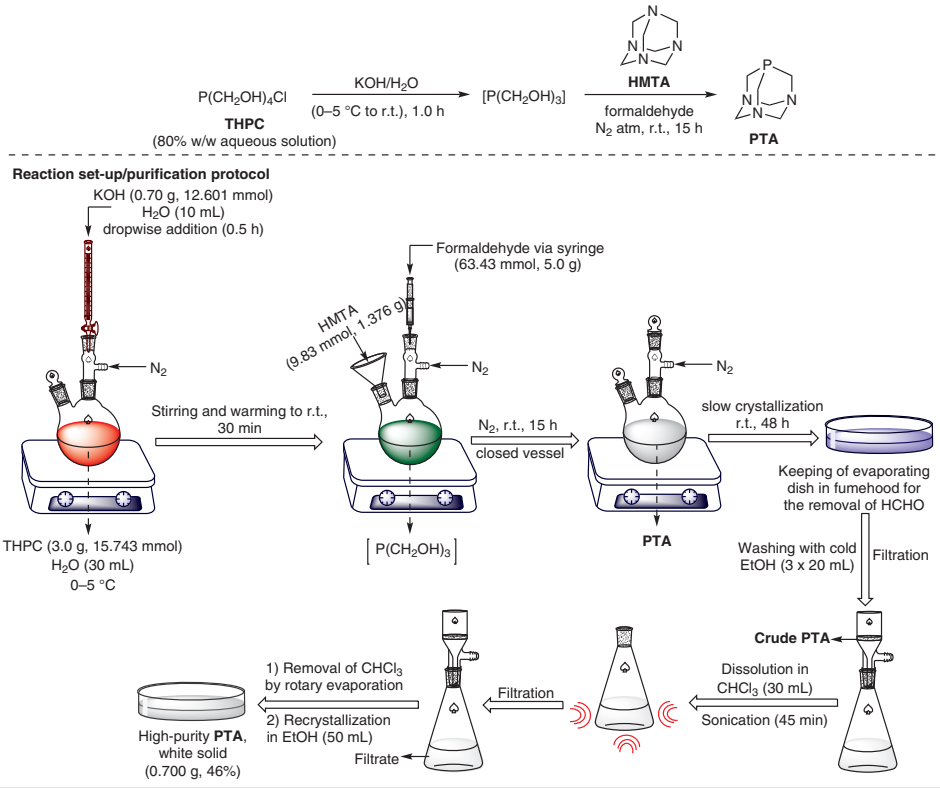
Formaldehyde (1250 mmol) and HMTA (200 mmol) were added to the reaction mixture containing the preformed tris(hydroxymethyl)phosphine and the resultant solution was left at room temperature for 12 hours. The resultant product (18.2 g) was a mixture of unreacted HMTA and PTA (80:20). It is important to note that the separation of PTA from HMTA was difficult due to similar polarities. All attempts to purify PTA by washing with different solvents (ethanol, methanol, acetone, or diethyl ether) or crystallization failed in our hands. Lower conversion of the HMTA to PTA could be attributed either to the incomplete conversion of THPC to tris(hydroxymethyl)phosphine or the replacement of nitrogen with phosphorus in the final step (release of NH_3 gas). The reaction could therefore be under a thermodynamically controlled equilibrium, although a change in temperature has no visible effect on the reactivity. The open-air reaction set-up could be yet another factor for the lower yield and it could be attributed to the release of NH_3 gas that is formed as a part of the synthetic process. To investigate such a possibility it was necessary to perform the reaction under an inert atmosphere and in a closed system (to create a positive pressure and assisting the thermodynamics of the reaction).



Scheme 4 Synthetic procedure using Daigle et al. method

The yield improvement experiment was performed on a small scale as illustrated in Scheme 5. First, a solution of THPC (3.0 g, 15.74 mmol) in water (30 mL) was placed in a double-necked round-bottomed flask purged with N_2 gas at ambient temperature. The reaction mixture was cooled (0–5 °C) and a solution of KOH (0.7 g, 12.60 mmol) in 10 mL deionized water was added over 30 minutes. The resultant solution was stirred for 30 minutes and allowed to warm to room temperature to generate the intermediate tris(hydroxymethyl)phosphine [$P(CH_2OH)_3$] that is used directly without isolation for the subsequent reaction. Next, HMTA (1.37 g, 9.83 mmol) was then added to the solution followed by formaldehyde (5.0 g, 61.43 mmol, 40%) addition via a syringe over 30 minutes. The resulting mixture was stirred under N_2 atmosphere at ambient temperature for 15 hours in a closed system (glass stopper).

Next, the reaction mixture was transferred to an open evaporating dish and placed in a fume hood for 48 hours for gradual evaporation of excess formaldehyde and water. This process promoted the crystallization of PTA. The solid product was filtered and washed with cold ethanol (3 × 20 mL) to furnish crude PTA. The solid material was suspended in $CHCl_3$ (30 mL) and the mixture sonicated for 45 minutes. Next, undissolved solids were filtered and the filtrate concentrated on a rotary evaporator. The residue was easily crystallized from EtOH (50 mL) offering high purity PTA



Scheme 5 Improved protocol for the synthesis of PTA

(confirmed by ^{1}H, ^{31}P NMR, and elemental analysis) in an overall 46% yield. This protocol was repeated to establish consistency and scalability (Table 2). PTA prepared on gram-scale (Table 2, entry 4) was found to be suitable for the large-scale synthesis of PTABS.

Table 2 Scale-Up Studies for PTA Synthesis

Entry	HMTA (mmol)	HMTA (amount, g)	Isolated PTA (amount, g)	Yield (%)
1	9.83	1.37	0.70	46
2	98.3	13.76	7.08	46
3	196.6	27.52	14.40	47
4	393.2	55.04	28.06	46

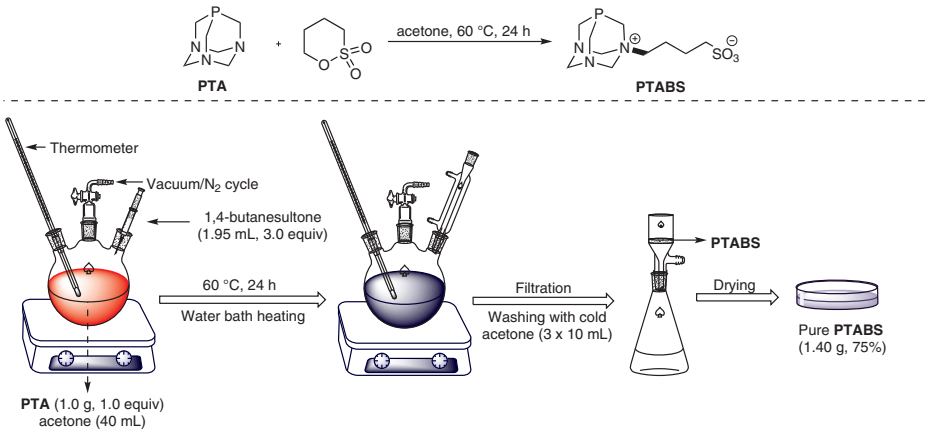
The original protocol for the synthesis of PTABS involved the reaction of PTA (1.0 equiv) with 1,4-butanesultone in acetone as a solvent at 60 °C for 24 hours.[30] The set-up for the protocol is illustrated in Scheme 6. The synthesis of PTABS was found to be reproducible on gram-scale (Table 3). An analytically pure sample of PTABS was obtained by the washing of the product with cold acetone (3 × 10 mL; Table 3, entry 1).

2.3 Multigram Scale-Up of Ruth Linker

With the gram quantity of PTABS in hand, the stage was set for performing the scale-up of Ruth linker. As summarized in Section 2.1 (Table 1, entry 3) identical conditions for the Heck alkenylation of 5′-*O*-DMTr-5-iodo-2′-deoxyuridine (**1**) with acrylamide linker **2** using $Pd(OAc)_2$/PTABS catalytic system were employed for scale-up experiments. To our delight, both experiments worked well furnishing high yield and excellent product purity (Table 4). After a successful scale-up of Heck reaction for the synthesis of Ruth linker precursor at 5.0 mmol, we decided to examine the developed protocol at the multigram level. Next, an experiment using 10 g of compound **1** was carried out furnishing identical yield of the desired product (74%, Table 4, entry 1). Lastly, we carried out the synthesis of Ruth linker on a 100 g scale using developed protocol of Pd/PTABS

Table 3 Scale-Up Study for PTABS Synthesis

Entry	PTA (mmol)	PTA (amount, g)	Isolated PTABS (amount, g)	Yield (%)
1	6.36	1.0	1.40	75
2	31.81	5.0	7.18	77
3	63.63	10	14.18	76



Scheme 6 PTABS synthesis procedure

Table 4 Multigram Scale-Up Synthesis of Ruth Linker[a]

Entry	Amount of SM 1 (mmol)	SM 2 (mmol)	$Pd(OAc)_2$ (g)	PTABS (g)	Amount of Product **A** in g (% isolated yield)	HPLC purity (%)
1	10 g (15.2)	16.7	0.068	0.178	9.4 (74)	96
2	100 g (152.3)	167.5	0.682	1.781	92 (70)	97

[a] Reaction conditions are the same as Table 1, entry 3: 1.0 equiv of **1**, 1.1 equiv of **2**, 2.0 mol% $Pd(OAc)_2$, 4.0 mol% of PTABS, Et_3N 2.0 equiv, MeCN as solvent (for entry 1, 50 mL and for entry 2, 1 L). All are isolated yields.

catalytic system and gratifyingly, the desired product was obtained in 70% isolated yield with >97% HPLC purity (entry 2).

3 Conclusion

In summary, we have reported herein a highly efficient palladium-catalyzed Heck alkenylation protocol for the synthesis of Ruth linker on a large-scale ideal for industrial use. Various reaction parameters were studied and optimized to accomplish both high yield and excellent quality required for conversion to an amidite. The salient features of this study encompass the reaction conditions that were gentle enough to tolerate an acid-labile 5′-*O*-DMTr and a base-labile amino-TFA protecting groups. This study clearly demonstrated the application of a highly active Pd/PTABS system where a reduction in the Pd concentration (2.0 mol%) was possible. The stability of the catalytic system eliminated the requirement of extra additive allowing the reaction to be completed in an exceptionally short period. The commercial viability of the protocol was achieved by the development of a column-free isolation protocol with an overall ~20% improvement in the yield of the Ruth linker (in comparison to existing protocols in academia or industry), purity of >97% and reproducibility of the protocol on large-scale. Additionally, we have reported an optimized and efficient scale-up protocol for PTA and PTABS ligand. This study paves the way for future process development of more complex molecules where late-stage Heck reaction could be executed under mild and efficient conditions.

All reactions were performed under a N_2 atmosphere using anhydrous solvents used without further purification, unless otherwise stated. The 5′-*O*-DMTr-5-iodo-2′-deoxyuridine (**1**) and alkene **2** were purchased from Sapala Organics Pvt. Ltd. and used without further purification. Reduced pressure distillations were performed between 250 and 25 mbar. HPLC spectra were obtained on Shimadzu HPLC (model no. LC-2030 C 3D plus) with a UV detector at 254 nm and Hypersil BDS C18 (250 × 4.6 mm, 5 µ) column at 35 °C; flow rate 1.2 mL/min.; mobile phase A: MeCN; B: 5 mM NH_4OAc in H_2O; run time 70 min.; 5 to 90% A over 70 min.; sample diluent, MeOH. NMR spectra recorded on NMR-500 MHz (JEOL) or an Agilent 500 MHz instrument and calibrated using residual undeuterated solvent as an internal reference ($CHCl_3$: ^1H NMR, δ = 7.26; ^{13}C NMR, δ = 77.16; DMSO: ^1H NMR, δ = 2.50, ^{13}C NMR, δ = 39.52). Standard abbreviations are used to explain ^1H NMR multiplicities. Reactions were monitored by TLC carried out on commercial silica gel plates (GF254) using UV light as visualizing agent. IR spectra were recorded on a PerkinElmer 16F PC FTIR spectrophotometer. LCMS were performed on Shimadzu LCMS spectrometer (model no. LCMS-2010 EV).

Synthesis of PTA

A clean and oven-dried double-necked round-bottomed flask equipped with a magnetic stirring bar was evacuated and flushed three times with N_2. Under a flow of N_2, THPC [tetrakis(hydroxymethyl)phosphonium chloride; 3.0 g, 9.83 mmol, 80% w/w aq solution]

was added, diluted with deionized H_2O (30 mL), and cooled in an ice bath. A freshly prepared solution of KOH (0.7 g, 12.60 mmol) in deionized H_2O (10 mL) was added dropwise under stirring. The reaction mixture was allowed to stir for 30 min and pH checked (~8) to confirm that the mixture was basic. Next, formaldehyde (10.88 g, 145 mmol, 40 %) and HMTA (1.37 g, 9.83 mmol) were added in one portion. The reaction mixture was allowed to stir overnight and then the solution was transferred to a large evaporating dish. The evaporating dish was placed in a fume hood (having a good chemical scrubber facility) with a draft of air to first allow the removal of formaldehyde. Slow evaporation over 2 days induced the crystallization of PTA preferentially over HMTA. The solid thus obtained was filtered and washed with cold EtOH (3 × 20 mL) to obtain the crude PTA, which was subjected to a series of purification steps. First, crude PTA was dissolved in $CHCl_3$ (30 mL) and the mixture was sonicated for 45 min. Second, the suspension was gravity-filtered to remove undissolved solids. Third, the filtrate was evaporated under reduced pressure and the residue crystallized from EtOH (50 mL) to provide PTA in an overall yield of 46% (700 mg) as a white crystalline solid; mp 243–247 °C (Lit.[47] mp 244–250 °C).

IR (ATR): 2899, 1653, 1413, 1294, 1241, 966, 794, 579 cm^{-1}.

^1H NMR (500 MHz, D_2O): δ = 4.41 (q, *J* = 12.9 Hz, 1 H), 3.86 (d, *J* = 9.0 Hz, 1 H).

^{13}C NMR{^1H} (126 MHz, D_2O): δ = 70.8, 47.8, 47.7.

^{31}P NMR (162 MHz, D_2O): δ = –98.61 (s).

ESI-MS (+ve): *m*/*z* calcd for $C_6H_{12}N_3P$ [M]: 157.15; found: 158.19 [M + H]$^+$.

The compound exhibited identical NMR data to previous reports.[49]

Synthesis of PTABS

An oven-dried 250 mL three-necked round-bottomed flask equipped with an additional funnel, a thermo-pocket, and a magnetic stir bar was evacuated and flushed with N_2 three times. To this flask were added PTA (1.0 g, 1.0 equiv) and acetone (40 mL) under N_2 atmosphere. The resulting mixture was stirred for 15 min at r.t. to obtain an almost clear solution. Next, 1,4-butanesultone (1.95 mL, 3.0 equiv) was added dropwise under N_2 atmosphere. The reaction mixture was heated for 24 h at 60 °C while stirring with a cooling condenser assembly. The mixture was cooled to r.t. and the solvent removed using cannula. The solid product was washed with acetone (3 × 10 mL) furnishing 1.4 g (75%) of PTABS as a white solid; mp 252–254 °C (Lit.[34] mp 252–255 °C).

IR (ATR): 3423, 3019, 2959, 1675, 1471, 1266, 1171, 1128, 1036, 991, 943, 922, 784, 652, 600 cm^{-1}.

^1H NMR (500 MHz, D_2O): δ = 4.93 (d, *J* = 11.7 Hz, 2 H), 4.75 (d, *J* = 11.7 Hz, 2 H), 4.55 (d, *J* = 13.7 Hz, 1 H), 4.39 (d, *J* = 13.7 Hz, 1 H), 4.28 (d, *J* = 5.7 Hz, 2 H), 3.91 (t, *J* = 13.9 Hz, 2 H), 3.80 (dd, *J* = 14.9, 8.9 Hz, 2 H), 2.91 (dd, *J* = 17.4, 10.3 Hz, 4 H), 1.86 (dt, *J* = 16.0, 7.9 Hz, 2 H), 1.72 (dt, *J* = 14.7, 7.4 Hz, 2 H).

^{13}C NMR{^1H} (126 MHz, D_2O): δ = 79.0, 69.5, 62.3, 53.2, 52.9, 49.9, 45.8, 45.7 (d, *J* = 20.9 Hz), 21.3, 18.2.

^{31}P NMR (162 MHz, D_2O): δ = –84.51.

Anal. Calcd for $C_{10}H_{22}N_3O_4PS$ (PTABS·H_2O): C, 38.58; H, 7.12; N, 13.50; S, 10.30. Found: C, 38.87; H, 6.86; N, 13.52; S, 9.96.

The compound exhibited identical analytical data to previous reports.[30]

Synthesis of Ruth Linker A

An oven-dried two-necked round-bottomed flask equipped with a magnetic stir bar was evacuated and flushed with N_2 three times were charged with $Pd(OAc)_2$ (4.49 mg, 2.0 mol%) and PTABS ligand (11.72 mg, 4.0 mol%) under N_2 atmosphere, followed by MeCN. The resulting catalyst solution was stirred at r.t. for 5 min and then 5′-*O*-DMTr-5-iodo-2′-deoxyuridine (656 mg, **1**; 1.0 mmol), Et_3N (202 mg, 2.0 mmol), and MeCN were added under N_2 atmosphere. The reaction mass stirred for 5 min and then alkene linker **2** (292 mg, 1.1 mmol) and MeCN were added The reaction mass was then stirred at 80 °C in preheated oil bath for 3 h. After the completion of reaction (monitored by TLC and HPLC, using 5% MeOH in CH_2Cl_2 eluent system), the reaction mass allowed to cool to r.t. and diluted with CH_2Cl_2, stirred for 10 min, and filtered on Celite pad to remove undissolved solid mass. The filtrate collected was concentrated on a rotary evaporator. The residue obtained was dissolved in CH_2Cl_2 and transferred to a separating funnel and washed with distilled H_2O. The organic layer was concentrated using a rotary evaporator under reduced pressure and the residue obtained was dissolved in CH_2Cl_2 and then precipitated using hexane, and the solid obtained was filtered on a Büchner funnel. This re-precipitation method was then repeated again to get the pure product **A** (95–97% HPLC purity) as an off-white solid; yield: 75% (596 mg). The same procedure was used for the scale-up studies (Table 1 and Table 4); mp 139–141 °C.

HPLC analysis: Hypersil BDS C18 (250 × 4.6 mm, 5 µ), MeCN/5 mM NH_4OAc in H_2O (gradient), flow rate 1.2 mL/min, 35 °C; t_R = 37.71 min.

IR (KBr): 3541, 3083, 2836, 2352, 2049, 1865, 1798, 1582, 1446, 1294, 1153, 981, 790, 674 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 11.60 (s, 1 H), 9.40 (s, 1 H), 8.02 (t, *J* = 5.5 Hz, 1 H), 7.94 (s, 1 H), 7.37 (d, *J* = 7.6 Hz, 2 H), 7.27 (m, 6 H), 7.20 (d, *J* = 7.6 Hz, 1 H), 7.08 (q, *J* = 14.9 Hz, 2 H), 6.87 (dd, *J* = 8.6, 8.5 Hz, 4 H), 6.16 (t, *J* = 6.5 Hz, 1 H), 5.28 (d, *J* = 4.8 Hz, 1 H), 4.23 (t, *J* = 5.5 Hz, 1 H), 3.88 (d, *J* = 4.8 Hz, 1 H), 3.71 (d, *J* = 4.8 Hz, 6 H), 3.17 (m, 4 H), 3.11 (q, *J* = 6.4 Hz, 2 H), 2.26 (m, 2 H), 1.44 (m, 4 H), 1.27 (s, 4 H).

$^{13}C\{^1H\}$ NMR (126 MHz, DMSO-d_6): δ = 165.5, 161.8, 158.1, 156.1 (q, *J* = 39 Hz, $COCF_3$), 149.3, 144.9, 142.7, 135.6, 135.5, 132.1, 129.6, 127.8, 127.7, 126.7, 122.1, 116.0 (q, *J* = 289 Hz, CF_3), 113.2, 109.4, 85.6, 85.5, 84.6, 70.2, 63.9, 54.9, 38.5, 29.5, 28.2, 26.0, 25.9.

ESI-MS (–ve): *m*/*z* [M] calcd for $C_{41}H_{45}F_3N_4O_9$: 794.31; found: 793.4.

The compound exhibited identical data to previous reports.[25]

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0040-1707260.

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