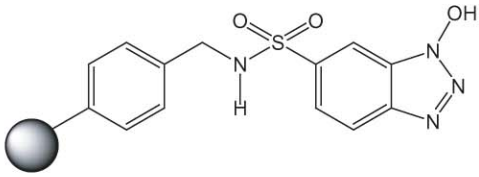
Technical Note 505

PS-HOBt(HL)

Resin-Bound Active Ester Reagent



Chemical Name: 1-Hydroxybenzotriazole-6-sulfonamidomethyl polystyrene

Resin Type: 1% Cross-linked poly(styrene-co-divinylbenzene)

Loading: 0.9–1.5 mmol/g (based on benzoylation of benzylamine)

Bead Size: 75–150 microns, 100–200 mesh (95% within)

Application: Active ester reagent; coupling of acids and amines; protecting group (Fmoc, CBz, Boc) transfer

Typical Acid Loading Conditions: 1.5 equiv of carboxylic acid, 4.5 equiv of DIC, 0.6 equiv of DMAP in a 4:1 DCM/DMF solvent mixture at room temperature for 2 hours.

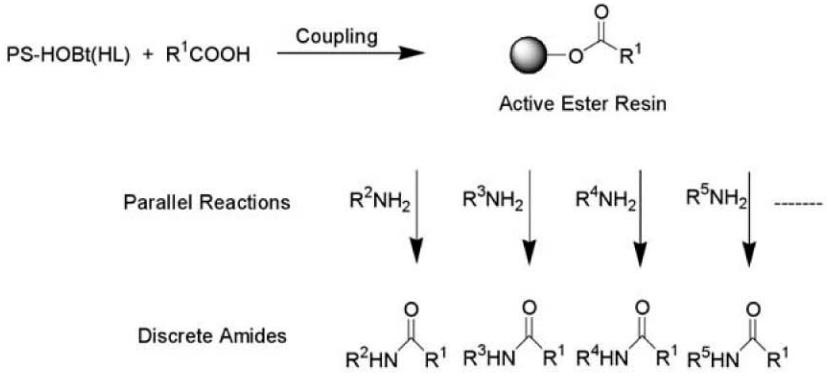
Typical Protecting Group Loading Conditions: 3 equiv of FmocCl or CbzCl and 5 equiv of Py in DCM at room temperature for 1 hour.

Compatible Solvents: DMF (7.5 mL/g), THF (4.8 mL/g), DCM (3.0 mL/g), and other solvents that swell gel-type polystyrene.

PROCEDURE FOR COUPLING ACIDS AND AMINES

PS-HOBt(HL) is a sulfonamide-linked, resin-bound equivalent of 1 hydroxybenzotriazole (HOBt).[1] PS-HOBt(HL) is used to generate bound HOBt active esters, which can either be made and used in-situ, or isolated and stored as stable intermediates. Treatment of bound HOBt ester with an amine leads to amide formation in generally high purity without the need for further purification.

To date, the recommended procedure for loading PS-HOBt has required PyBroP as the coupling agent. We have developed an improved loading procedure based on the use of diisopropylcarbodiimide and DMAP. The new procedure has proven to be much more reliable, efficient and cost effective. It requires fewer equivalents of carboxylic acid and does not require a double coupling as is the case when PyBrOP is used.



Scheme 1. Parallel Synthesis of Amides with PS-HOBt(HL) Active Esters

PS-HOBt(HL)

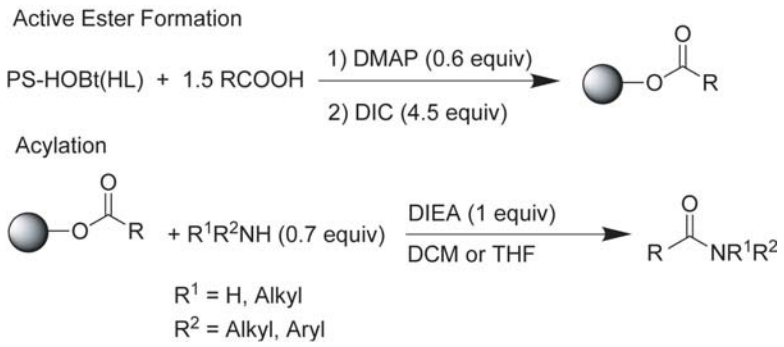
PART NUMBER	QUANTITY
800417	10 g
800418	25 g
800419	100 g
800420	1000 g

Biotage

Resin-bound active esters offer some unique advantages for parallel amide synthesis. The resin-bound active esters of PS-HOBt(HL) can be prepared using the coupling protocol and purified by solvent washes. Amide formation is accomplished by simply adding the appropriate amine to the active ester resin. By using the amine as the limiting reagent, acylation of the amine generates the amide as the sole compound in solution. Only filtration and concentration are required to isolate the product. A series of resin-bound HOBt active esters can be prepared in bulk from PS-HOBt(HL) and split into individual reactors for reaction with amine (**Scheme 1**). Depending on the stability of the bound active ester, long term storage is possible and the resin can be used as required to make amides. The stability of the bound active ester is dependent on the structure of the corresponding acid.

Formation of active esters of PS-HOBt(HL) was investigated using statistical design of experiments (DoE) in conjunction with the Trident® Library Synthesizer.[2] This work demonstrated optimal conditions for active ester formation and used 1.5 equiv of carboxylic acid, 4.5 equiv of DIC, 0.6 equiv of DMAP, and a 4:1 DCM/DMF solvent mixture at room temperature for 2–3 h (**Scheme 2**). Order of addition proved to be an important variable. DMAP and the carboxylic acid were mixed with PS-HOBt(HL), followed by addition of DIC. The purpose of including DMF as a cosolvent is to improve the solubility of the carboxylic acid component. It is preferable to keep the DMF composition in the reaction mixture to a minimum (< 20%), since it compromises the level of active ester formation. If more DMF is required to dissolve the carboxylic acid, up to 50% DMF can be used, however, the loading level will be reduced.

The formation of PS-HOBt(HL) active esters from five carboxylic acids using the DIC/DMAP protocol afforded resin-bound active esters with loading levels ranging from 65–96% of theory (**Table 1**). The best results were obtained with aromatic and aliphatic carboxylic acids. In addition to DIC/DMAP, 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP) was found to be an alternative coupling agent for PS-HOBt(HL) active ester formation.[3]



Scheme 2. Amide Synthesis with PS-HOBt(HL)

Table 1. PS-HOBt(HL) Active Ester Formation Using DIC/DMAP

Acid	Loading (mmol/g)			% Loading
	PS-HOBt(HL)	Active Ester Theoretical	Active Ester Measured	
Benzoic acid	1.0	0.91	0.87	96
Cyclopentyl propionic	1.0	0.89	0.85	96
Cinnamic acid	1.0	0.88	0.65	73
Quinaldic acid	1.0	0.87	0.56	65
Boc-Phe	1.0	0.80	0.56	70

Amide formation is effected by mixing the PS-HOBt(HL) ester resin with 0.5–0.7 equiv of amine, and 1 equiv of DIEA in DCM, THF, or DCE. The reaction takes place at room temperature with both aliphatic and aromatic amines, although less nucleophilic aromatic amines may require 65 °C. Likewise, other non-nucleophilic amines, e.g. aminothiazoles, can react at elevated temperatures to afford amides, depending on the amine structure. Products are isolated by filtration and concentration. The acylation reaction has been successfully carried out with benzyl amine in wet DCM, demonstrating that this step is fairly robust to adventitious moisture. The use of DIEA is optional for aliphatic amines, however its use often results in higher yields. DIEA is required for less nucleophilic amines, such as anilines, and is readily removed under reduced pressure. Acylation can also be performed with PS-HOBt(HL) active ester as the limiting reagent, in which case excess amine is scavenged from the product with PS-Isocyanate.

Active esters from a set of carboxylic acids that included aromatic, aliphatic, cinnamic, and amino acids were formed from PS-HOBt(HL) using the DIC/ DMAP protocol. These were used to acylate benzyl amine and 1-phenylpiperazine at room temperature, and aniline at 63 °C (**Table 2**). The amines were used as the limiting reagent at 0.7 equiv relative to the loading of the starting PS-HOBt(HL) resin (1.0 mmol/g). Isolation by filtration with solvent removal under reduced pressure afforded the amide products. The aromatic, aliphatic, and cinnamic acids afforded high purity products in good to excellent yield. In some cases, excess 1-phenylpiperazine was present in the product and was removed by scavenging with PS-Isocyanate. Amides from Boc-ala, Boc-phe and 4-bromophenylacetic acid generally afforded modest yields of amide.

An alternative coupling reagent for preparation of PS-HOBt(HL) active esters is 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP).[3] Comparison of coupling conditions using 1.5 and 4 equiv of BEP showed 1.5 equiv to give better results. A limited set of carboxylic acids was converted to PS-HOBt(HL) active esters using BEP and then used to acylate benzyl amine (**Table 3**) The results demonstrate that BEP is a viable alternative to the DIC/DMAP procedure and may be preferred for some substrates as indicated by the improved yield of amide obtained from 4-bromophenylacetic acid and Boc-phe.

Table 2. Synthesis of Amides Using PS-HOBt(HL)

Acid	Benzyl Amine rt		1-Phenylpiperazine rt		Aniline 63 °C	
	%Yield	%Purity	%Yield	%Purity	%Yield	%Purity
Benzoic	93	92	87	96	86	94
2-Naphthoic	92	89	82	93[a]	92	87
4-biphenylcarboxylic	90	92	80	96	82	90
Cinnamic	73	98	65	97	55	95
3-Phenylpropionic	75	99	76	99[a]	90	95
Cyclohexanecarboxylic	92	96	92	99	83	99
Cyclopentylpropionic	80	98	77	99	90	99
Boc-ala	56	93	64	99[a]	61	97
Boc-phe	54	98	64	99[a]	54	97
4-Bromophenylacetic	39	88	88	83[a]	40	95

[a]Sample incubated with PS-Isocyanate; approximately 1 equiv relative to the PS-HOBt(HL)

Table 3. Synthesis of Amides from Benzyl Amine Using BEP for Formation of PS-HOBt(HL) Active Esters

Acid	% Yield	% Purity
2-Naphthoic	98	95
3-Phenylpropionic	60	97
Boc-ala	34	90
Boc-phe	74	97
4-Bromophenylacetic	72	88

The stability of isolated PS-HOBt(HL) active esters derived from the carboxylic acids in **Table 2** has been monitored by measuring amide yield and purity obtained after acyl transfer to benzyl amine over time. At 4 °C over a one month test period, benzoic, quinaldic and cinnamic bound esters showed excellent stability, while 3-phenylpropionic and Boc-phe esters were observed to undergo approximately 30% decrease in loading. The decrease in loading for 3-phenylpropionic and Boc-Phe esters was more rapid at room temperature, demonstrating that low temperature storage is beneficial. Active esters of benzoic and quinaldic acids were stable at room temperature, and in the case of benzoic acid, a sample was successfully stored for over 3 months with no degradation. In the case of acids that underwent some degradation on storage, the resin samples were washed with THF prior to reaction with amine, and afforded high purity products upon cleavage. The results demonstrate that most active esters can be prepared in bulk and stored cold for future use, however, the shelf-life can vary from days to months depending on carboxylic acid structure.

REPRESENTATIVE PROCEDURES

Procedure for Making PS-HOBt(HL) Active Esters using DIC/DMAP:

The solvents and reagents for preparing PS-HOBt(HL) active esters should be dry and care should be taken to avoid contamination by atmospheric moisture. To 150 mg of PS-HOBt(HL) resin (1.0 mmol/g, 0.15 mmol) was added 2 mL of a 0.045 M solution of DMAP in DCM (0.090 mmol) and 0.6 mL of a 0.38 M carboxylic acid solution in DMF (0.23 mmol). The mixture was shaken/agitated briefly. This was followed by the addition of 0.4 mL of 1.65 M DIC in DCM (0.66 mmol) and the solution was mixed for 3 h at room temperature. The mixture was filtered and the resin was washed with DMF (3 x 3 mL), DCM (3 x 3 mL), DMF (3 x 3 mL), and THF (3 x 3 mL) and dried to afford purified resin-bound HOBt active ester. Note: *The cosolvent composition should be ~20% DMF. If more DMF is required to dissolve the acid, up to 50% DMF can be used, however carboxylic acid loading is likely to be reduced.*

Procedure for Loading Carboxylic Acids at the 4 g Scale:

Larger amounts of PS-HOBt(HL) active esters were prepared by analogy with the small scale preparation using a Quest® 205 synthesizer. To 3.75 g of PS-HOBt(HL) (1.0 mmol/g, 3.75 mmol) in a Quest 205 reaction vessel, was added 50 mL of a 0.045 M solution of DMAP in DCM (2.25 mmol) and 15.0 mL of a 0.38 M of carboxylic acid solution in DMF (5.63 mmol). The mixture was mixed for 15 minutes, followed by addition of 10.0 mL of 1.65 M DIC in DCM (16.5 mmol). The solution was then

mixed for 3 h at room temperature. The mixture was filtered and washed with the same solvents as the small scale reactions (60 mL of solvent) and dried to afford purified resin-bound HOBt active ester.

Procedure for Loading Carboxylic Acids with BEP:

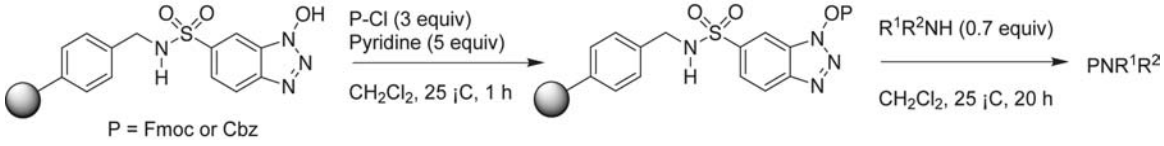
2-Bromo-1-ethylpyridinium tetrafluoroborate (BEP) was prepared according to the literature procedure.[3] To 150 mg of PS-HOBt(HL) resin (1.0 mmol/g, 0.15 mmol) was added 1.5 mL of a 0.16 M solution of BEP in DCM (0.024 mmol) and 0.6 mL of a 0.38 M carboxylic acid solution in DMF (0.23 mmol). The mixture was shaken/agitated briefly. This was followed by the addition of 1.0 mL of 0.53 M solution of DIEA in DCM (0.53 mmol) and the solution was mixed for 3 h at room temperature. The mixture was filtered and the resin was washed with DMF (3 x 3 mL), DCM (3 x 3 ml), DMF (3 x 3 mL), and THF (3 x 3 mL) and dried to afford purified resin-bound HOBt active ester.

Representative Procedure for Amide Synthesis:

To PS-HOBt(HL) active ester resin (0.15 mmol) was added a mixture of amine (0.07 mmol) and DIEA (0.1 mmol) in DCM or THF (2 mL) and the mixture was stirred at 25 °C for 3 h. The solution was filtered, and the resin rinsed with DCM or THF (3 x 3 mL). The combined filtrate was concentrated to afford the desired amide. If the PS-HOBt(HL) active ester resin has been stored for an extended period, it is recommended that it be washed 2–3 times with DCM or THF prior to use.

PROCEDURE FOR LOADING PROTECTING GROUPS

PS-HOBt(HL) can be used as an effective activating reagent for transfer of protecting groups, e.g.,Fmoc and Cbz, to amines.[1]



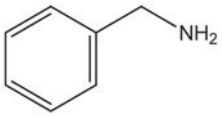
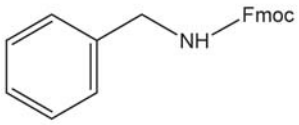
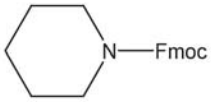
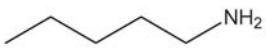
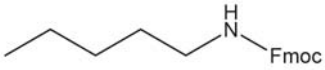
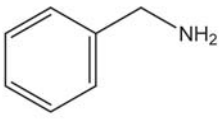
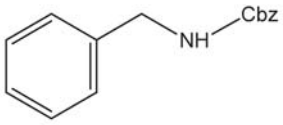
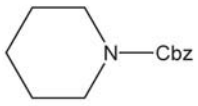
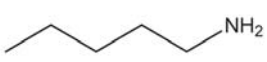
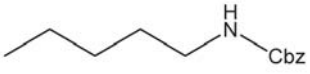
REPRESENTATIVE PROCEDURE

Protection of Amines as Fmoc Derivatives (Entry 1, Table 4):

225 mg of PS-HOBt resin (0.9 mmol/g, 0.2 mmol) and a solution of FmocCl (127 mg, 0.49 mmol) in 1.1 mL DCM were added to a reaction vessel. A solution of pyridine (65 mg, 0.82 mmol) in 1.2 mL DCM was then added. The mixture was stirred for 1 h at 25 °C.

The reaction mixture was drained and the resin washed with DCM (3 x), DMF (3 x), DCM (3 x) and diethyl ether (3 x). A solution of benzyl amine (14 mg, 0.13 mmol) in 3.2 mL DCM was added, and the mixture was stirred for 20 h at 25 °C. Finally, the solution was filtered into a pre-weighed vial (washed 3 x with DCM) to rinse product from the resin). The solvent was then concentrated to give Fmoc-benzylamine in 78% yield (HPLC purity 99%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ7.75 (d, 2 H, Ar-H), 7.60 (d, 2H, Ar-H), 7.45-7.20 (m,9 H, Ar-H), 5.10 (s, 1 H, N-H), 4.48 (d, 2 H, CH_2), 4.40 (d, 2 H, CH_2), 4.25 (t, 1 H, CH); ^{13}C NMR ($CDCl_3$, 75 MHz): d 156.40, 143.92, 141.34, 138.38,127.65, 127.52, 127.03,125.00, 119.96, 66.69, 47.32, 45.12 ppm.

Table 4: Protection of Amines Using PS–HOBt Resin

Entry	Protecting Group P–Cl	Amine R^1R^2NH	Protected Amine PNR^1R^2	Yield %	Purity %
1	Fmoc–CL			78	99
2	Fmoc–CL			75	100
3	Fmoc–CL			76	100
4	Cbz–CL			87	97
5	Cbz–CL			42	95
6	Cbz–CL			70	95

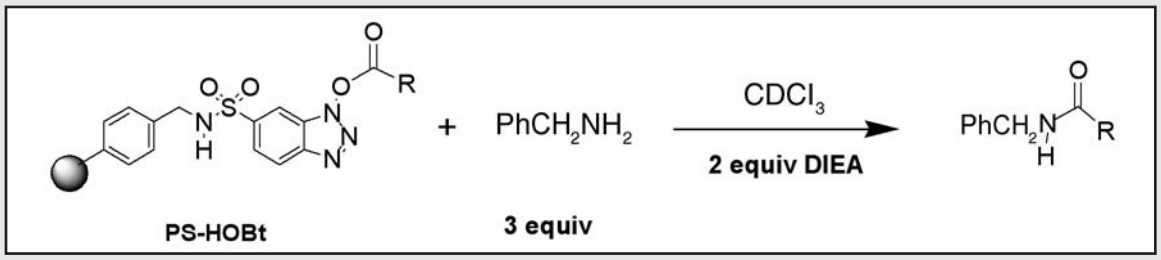
DETERMINATION OF PS-HOBt(HL) ACTIVE ESTER FORMATION USING ^{1}H NMR.

We have developed a convenient method for measuring the amount of active ester formed after reaction of PS-HOBt(HL) with the carboxylic acid. This value is used to determine the correct amounts of bound active ester and amine to use in amidation reactions.

The percent active ester formation (mmol of ester per gram of ester resin) can be determined by acylating benzylamine with the PS-HOBt(HL) active ester as the limiting reagent in an NMR tube. The loading of the PS-HOBt(HL) is calculated from the mmol of benzyl amide formed, as determined by comparison of the integration of benzylamine CH_2 (s, 3.8 ppm) and the benzyl amide CH_2 (d, 4 to 5 ppm) in a ^{1}H NMR spectrum.

Typically, about 40 mg of active ester resin (~0.04 mmol) is transferred to a 5 mm NMR tube and mixed with 1 mL of $CDCl_3$ solution containing 0.12 mmol of benzylamine and 0.08 mmol of DIEA. The septum-capped tube is agitated on a rotary mixer for 3 h before obtaining the proton NMR of the solution. Since the resin floats in $CDCl_3$ solutions, it does not interfere with the measurement. The loading is calculated from the exact weight of the resin, amount of amine added and the integration values of the two benzylic resonances. The ^{1}H NMR method generally gives a value that is slightly higher (~10%) than a direct cleavage. This level of accuracy is sufficient to calculate the amount of amine to add in reactions with the bulk PS-HOBt(HL) active ester. The procedure is also applicable to most PS-TFP esters, with the exception that longer acylation times (16 h) are required.

It is important to add DIEA to minimize selective partitioning of the benzylamine in the bead, caused by protonation by free HOBt alcohol groups.When there are interferences due to resonances from the carboxylic acid substrate, the integration values can be corrected before the calculation, since the number of interfering protons from the substrate is often known. This method can be used to ascertain the reactivity of an active ester by monitoring the formation of the benzyl amide over time.



PROCEDURE

A. NMR sample preparation and analysis

1) Scoop a small amount (~0.04 to 0.05 g,~0.4 mmol) of dried active ester resin directly into a tared and labeled 5 mm NMR tube. Record the actual resin weight and cap the tube with a rubber septum (Aldrich Chem. Cat. # Z10,070-6).

2) Prepare 10 mL of $CDCl_3$ cleavage solution (enough for ~10 measurements) in a 20 mL scintillation vial as follows:

i. Pass ~ 3 mL of benzylamine through a 500 mg silica plug, discard the first 1 mL and collect the remaining for the next step.

ii. Weigh approximately 0.128 g of purified benzylamine into the open scintillation vial, record the weight.

iii. Weigh approximately 0.103 g of DIEA into the same vial, record the weight.

iv. Cap the scintillation vial with a septum cap, tare the capped vial, then syringe in 10 mL of $CDCl_3$ through the septum. Record the actual $CDCl_3$ weight. Mix well.

3) With a syringe and 25-gauge needle, withdraw the cleavage solution and deliver 1.0 mL into the tared, septum capped NMR tube containing the resin. Insert a needle through the septum briefly to vent the tube. Record the weight of $CDCl_3$ solution.

4) Mix the resin and cleavage solution in the NMR tube. Tap gently if necessary.

5) Tape the NMR reaction tube onto a rotary shaker and rotate at low speed to move the mixture back and forth along the tube's length.

6) After rotating overnight at 25 °C, let the tube stand until most of the resin floats to the top of the liquid column.

7) Obtain ^{1}H NMR of the mixture. Cleavage kinetics can also be investigated by obtaining the ^{1}H NMR at different time intervals

8) Expand the region between 3.6 to 5 ppm and integrate the benzyl-H of the unreacted benzylamine (singlet, 3.8 ppm) and the benzyl-H of the resulting amide product (doublet, 4 to 5 ppm).

B. Loading Calculation

1) Calculate the mmol of benzylamine added into the resin: mmol amine added = [(Wt of $CDCl_3$ soln added to resin x Wt of benzylamine in $CDCl_3$ soln)/(total Wt of $CDCl_3$ solution)] x 0.10716

2) Calculate the mmol of amide formed using the proton integration values. Taking into account any interfering signal(s): mmol amide formed = (Amide-H integration x mmol amine added)/ (Amine-H integration + Amide-H integration)

3) Calculate the loading (mmol/g) of the active ester resin: Loading = mmol amide formed/g of active ester resin.

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