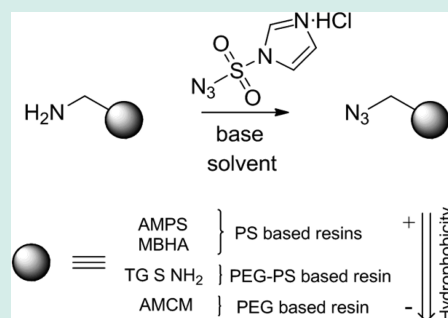


Imidazole-1-sulfonyl Azide-Based Diazo-Transfer Reaction for the Preparation of Azido Solid Supports for Solid-Phase Synthesis

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S Supporting Information

ABSTRACT: An efficient, standard, mild, and copper-free imidazole-1-sulfonyl azide hydrochloride-based diazo-transfer method was implemented in a set of four resins that cover a broad range of hydrophobicity. The imidazole-1-sulfonyl azide hydrochloride is easily prepared/commercially available, stable upon storage at 4 °C, and proved to be a suitable alternative to triflyl azide for diazo-transfer reactions in amine functionalized resins. We have successfully applied the azido resins for the conjugation of a TFA-labile Wang-type linker using Click Chemistry.



KEYWORDS: imidazole-1-sulfonyl azide hydrochloride (ISA-HCl), diazo-transfer, solid phase, azido resins, Click Chemistry

Solid-phase synthesis (SPS) has become a method for the synthesis of a wide range of organic molecules, including peptides,¹ nucleotides,² and small organic compounds.³ In this regard, solid supports are one of the most important tools for the preparation of libraries and functionalized supports, such as those used for affinity chromatography, which have a broad application in combinatorial sciences. Among the variety of solid supports that are commercially available, polystyrene (PS), polyethyleneglycol grafted on polystyrene (PEG-PS), and polyethyleneglycol (PEG) are the most commonly used resins.^{4,5} [The terms “solid support” and “resin” are usually interchangeable in this field. In this manuscript, solid support denotes the chemical structure of the base polymer (i. e., polystyrene, polyethyleneglycol, polyethyleneglycol grafted on polystyrene, etc.), whereas the resin indicates the solid support functionalized with a cleavable linker, which will release the molecule at the end of the synthesis. The functionalization is performed through a linker, which is defined as an insoluble, immobilizing group for SPS.] The wide variety of building blocks and nonintegral linkers are coupled to the resin mainly through an amide bond because most commercial solid supports are functionalized with primary amines. [Linkers can be divided into the following: (i) *integral linkers*, in which the linker is part of the solid support core, and (ii) *nonintegral (or grafted) linkers*, where the linker is attached to a conveniently functionalized resin core.⁵] Of note, the cleavage of the compounds attached to the resin through some of these nonintegral linkers leads to side products, which reduce the final purity and yield.⁶

During the past decade the Click Chemistry concept, and especially the copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction between terminal alkynes and organic azides, has been adapted to many areas of chemistry, from material sciences to drug discovery.^{7–9} The CuAAC yields 1,4-disubstituted 1,2,3-triazoles and exhibits remarkably broad scope and selectivity.¹⁰ In the SPS field, the CuAAC reaction has been applied to the head-to-tail cyclodimerization of resin-bound oligopeptides,¹¹ the synthesis of triazoles,¹² and the preparation of complex peptides through side-chain cyclization.¹³ In addition, it has been shown that 1,4-disubstituted 1,2,3-triazoles are a suitable alternative to anchor *nonintegral* linkers to the solid support.¹⁴ Very recently, our group reported a new Wang-like resin, where the linker is anchored to the solid support through a stable triazole moiety prepared via CuAAC.¹⁵

The introduction of azide groups on solid phase has been accomplished mainly through direct nucleophilic displacement of chloromethyl derivatized solid supports by the azide ion.^{16,17} Some of these methods proceed sluggishly under the harsh conditions used and lack reproducibility. To overcome these limitations, a diazo-transfer-based reaction using triflyl azide (TfN₃) was developed to convert the amine to azide on solid-phase.² However, TfN₃ is unstable and potentially explosive, requiring in situ preparation. Very recently, Hansen et al. reported the use of imidazole-1-sulfonyl azide hydrochloride

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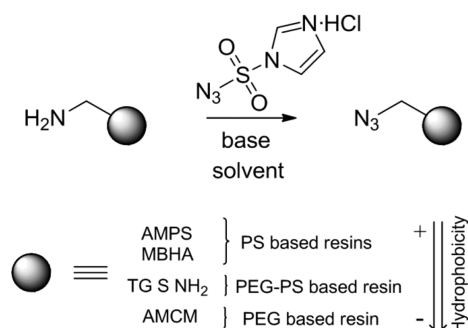
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(ISA·HCl) as the diazo-transfer reagent for the efficient solid-phase preparation of azido peptides.¹⁸

On the basis of these observations and considering that ISA·HCl is stable, safe to handle, and synthetically highly accessible/commercially available diazo-transfer agent,^{19–21} here, we carried out a systematic study to establish a standard, efficient and mild protocol to prepare azido-solid supports. In this regard, we attempted to assess the scope and limitations of the diazotization reaction in several resins, including two types of polystyrene, namely, aminomethyl polystyrene (AMPS) and 4-methylbenzhydrylamine polystyrene (MBHA), a PEG-PS (Tentagel S NH₂, TG S NH₂), and a totally PEG resin (aminomethyl-ChemMatrix, AMCM) (Scheme 1). These four

Scheme 1. ISA·HCl-Based Diazo-Transfer Reaction on Solid Phase



solid supports cover a broad range of hydrophobicity, thus allowing the optimization of a set of conditions applicable to another solid supports of similar characteristics.

In a first approach, we screened a wide range of conditions to determine the most efficient procedure. The reaction was monitored by the colorimetric Kaiser test,²² quantitative ninhydrin analysis,²³ the reduction/ninhydrin test,²⁴ and IR spectroscopy.

Given the hydrophobic nature and swelling properties of PS, solvents, such as MeOH, MeCN, DMF, and DMSO, were tested (Table 1). Efficient conversions were achieved using 3 equiv of ISA·HCl, 9 equiv of base (DIEA), and 0.01 equiv of CuSO₄ as catalyst in either CH₃CN (Table 1, entry 5), DMF (Table 1, entry 10), or DMSO (Table 1, entry 13) as solvents. Interestingly, near quantitative conversions (~95%) in DMSO were achieved under copper-free conditions, thereby confirming previous findings showing efficient noncatalyzed diazo-transfer reaction with ISA·HCl (Table 1, entry 14).¹⁸ Using K₂CO₃ as base in MeOH or DMSO, only low conversions were detected (data not shown). This finding could be attributed to only partial dissociation of the base under these nonaqueous conditions, which would result in incomplete neutralization of the diazotransfer reagent hydrochloride salt and protonation of the more basic amine, with the consequent reduction of efficiency. Solvents such as DCM or *N*-methylpyrrolidone led only to poor conversions because of the low solubility of ISA·HCl on these solvents.

PEG-PS (TG S NH₂) and PEG-type resins (AMCM) show good swelling properties in a wide range of solvents, including water. Encouraged by previous results of ISA·HCl diazo-transfer reactions under aqueous conditions,^{18,25,26} we explored the viability of the amine to azide conversion on these resins using water as a solvent. The reaction was carried out with 3 equiv of ISA·HCl and 4.5 equiv of K₂CO₃ as base. Near

Table 1. Optimization of Diazo-Transfer Conditions on PS-Type Resins (AMPS and MBHA), Using ISA as Diazo-Transfer Reagent

entry	reaction conditions				N ₃ conversion (%) ^a	
	ISA·HCl (equiv) ^b	base (equiv) DIEA	catalyst (equiv) CuSO ₄	solvent	resins	
					AMPS ^c	MBHA ^d
1	3	9	0.01	MeOH	93	65
2	3	9		MeOH	75	54
3	7	9		MeOH	69	65
4	7	14		MeOH	84	62
5	3	9	0.01	MeCN	94	94
6	3	9		MeCN	85	43
7	7	9		MeCN	75	35
8	7	14		MeCN	91	68
9	3	4.5		DMF	29	41
10	3	9	0.01	DMF	85	94
11	7	14		DMF	80	89
12	3	6		DMSO	88	95
13	3	9	0.01	DMSO	98	97
14	3	9		DMSO	94	95
15	7	14		DMSO	94	96

^ammol/g of N₃ was calculated from the difference between mmol/g of NH₂ of unmodified and modified resin at 2 h by quantitative ninhydrin analysis. ^bISA·HCl = imidazole-1-sulfonyl azide hydrochloride. ^cAMPS resin (ρ = 0.72 mmol/g). ^dMBHA resin (ρ = 0.63 mmol/g).

quantitative conversions were obtained in the absence or presence of 0.01 equiv of CuSO₄ (Table 2, entries 1 and 3,

Table 2. Optimization of Diazo-Transfer Conditions on PEG-Based Resin (AMCM) and PEG-PS-Based Resin (TG S NH₂) Using ISA·HCl as Diazo-Transfer Reagent

entry	reaction conditions				N ₃ conversion (%) ^a	
	ISA·HCl (equiv) ^b	base (equiv) K ₂ CO ₃	catalyst (equiv) CuSO ₄	solvent	resins	
					TG S NH ₂ ^c	AMCM ^d
1	3	4.5		H ₂ O	>99	>99
2	7	4.5		H ₂ O	93	88
3	3	4.5	0.01	H ₂ O	93	94

^ammol/g of N₃ was calculated from the difference between mmol/g of NH₂ of unmodified and modified resin at a 2 h by quantitative ninhydrin analysis. ^bISA·HCl = imidazole-1-sulfonyl azide hydrochloride. ^cTG S NH₂ resin (ρ = 0.25 mmol/g). ^dAMCM resin (ρ = 0.35 mmol/g).

respectively). The increase of the amounts of ISA·HCl to 7 equiv lead to a slight diminution in the efficiency of the reaction (Table 2, entry 2). Analysis of the pH of the reaction showed that it is necessary a pH > 9 (or >pK_a of the amine) in order to reach high conversions.

Kinetic analysis of the diazotization reaction (Table 1, entry 14, and table 2, entry 1) showed that the reaction reached conversions of ~90% during the first hour for AMPS, TG S NH₂, and AMCM resins, whereas for MBHA this conversion is slightly lower (~80%). Additionally, in order to corroborate azide formation, elemental analysis of nitrogen was performed for the azido-resins prepared in the highest yielding conditions (Table 1, entry 14 for PS, and Table 2, entry 1 for PEG-PS and

Scheme 2. Click Reaction on Azide-Functionalized Solid Supports

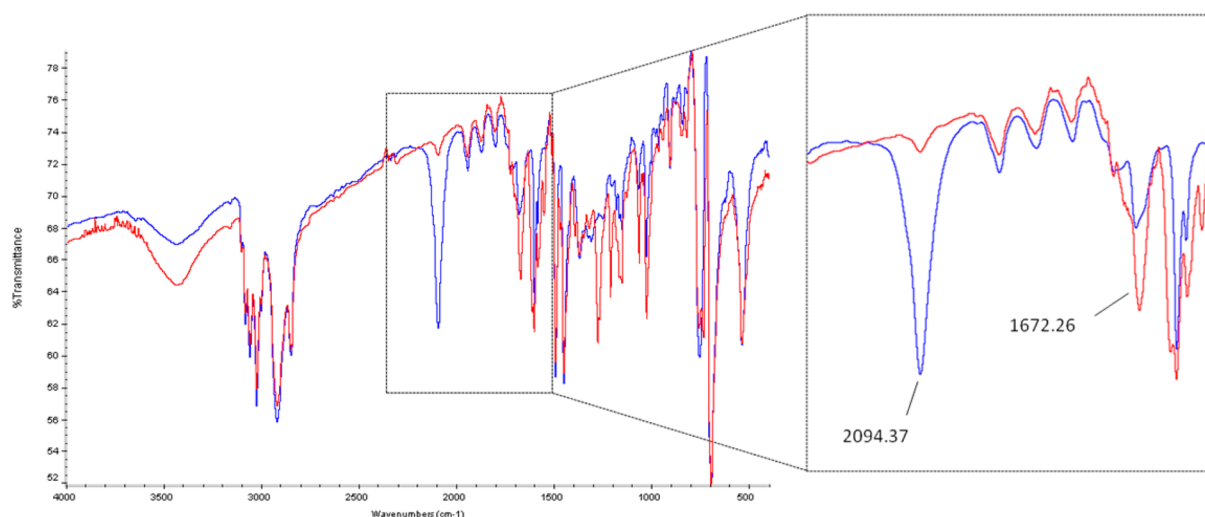
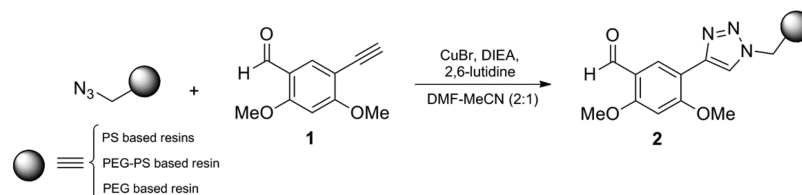


Figure 1. IR spectra of azide AMPS (blue line) and 2 (red line).

PEG resins). The results were consistent with previous quantification in all azido-resins (see Supporting Information (SI)).

To compare the efficiency of ISA·HCl and TfN₃ as diazo-transfer reagents, we prepared the four azido-resins using the reported TfN₃-based method.² In all cases, the amine to azide conversion was >90%. Although these results were similar to those obtained with ISA·HCl, it is important to mention that ISA·HCl is chemically more stable and safer, and therefore suitable for a large-scale preparation. In contrast, TfN₃ is highly explosive and difficult to isolate, thus hindering its use in high purity and at known stoichiometry.

The azide functionalization of the PS resins (AMPS and MBHA), PEG-PS resin (TG S NH₂), and PEG-based resin (AMCM) allows the robust incorporation of versatile linkers conveniently functionalized with an alkyne group. In order to demonstrate this approach, we attached the CHO-BTL linker (1) via copper-catalyzed azide–alkyne cycloaddition (CuAAC) on azide resin-based PS (AMPS), PEG-PS (TG S NH₂), and PEG (AMCM) resins. We recently reported that this linker is an alternative to Wang linker for the synthesis of C-terminal peptide acids.¹⁵

The CHO-BTL linker was loaded onto the azide resins via CuAAC to render CHO-BTL resins (2), following our previously reported procedure (Scheme 2).¹⁵ In general, these reactions were carried out with sodium ascorbate, 2,6-lutidine, DIEA, CuBr in a mixture DMF/ACN as solvents for 6 h at room temperature. The progress of CuAAC was monitored using IR spectroscopy, through the loss of the azide band at 2094 cm⁻¹ and the appearance of a new vibrational mode at 1672 cm⁻¹, indicative of the aldehyde group (Figure 1). ¹H-NMR of the starting azide resin and (2) corroborated the

linker coupling on the resin via Click Chemistry reaction (see SI).

In summary, here we have developed an efficient, standard, and mild ISA·HCl-based and copper-free diazo-transfer method for PS-(AMPS and MBHA), PEG-PS-(TG S NH₂), and PEG-(AMCM) type resins. For PS-based resins, 3 equiv of ISA·HCl, and 9 equiv of DIEA in DMSO as solvent were the optimal conditions to obtain a near quantitative amine to azide conversion. For the more hydrophilic TG S NH₂ and AMCM resins, 3 equiv of ISA·HCl, and 4.5 equiv of K₂CO₃ in water as a solvent provided the best results. The method developed here should be applicable to a broad range of solid phase resins and be used for subsequent Click Chemistry solid phase reaction, as well as in the preparation of azido-peptides.

EXPERIMENTAL PROCEDURES

Solid Phase Diazo-Transfer General Procedure. Fifty milligrams of either AMPS, MBHA, TG S NH₂, or AMCM in a polypropylene syringe were washed with DCM and DMF (3 × 5 mL), TFA 1% in DCM (5 × 1 mL × 1 min each), DCM (3 × 5 mL), DIEA 5% in DCM (5 × 1 mL × 1 min each), and finally DCM. The four resins were swollen with the selected solvent (See Table 1 and 2) for the experiment and the swelling solution was filtered. The azido transfer reactions were carried out using imidazole-1-sulfonyl azide hydrochloride (ISA·HCl) (3 or 7 equiv), previously synthesized following the reported procedure,²⁰ K₂CO₃ (4.5 equiv) or DIPEA (6, 9, or 14 equiv) as a base, in 1 mL of solvent (MeOH, MeCN, DMF, DMSO for PS-based resins; and H₂O for PS-PEG- and PEG-based resins), and the reaction was gently stirred for 2 h at 25 °C. The final azido resins were washed with MeOH, DMF, and DCM and dried in vacuum. The conversion percentage was

determined by ninhydrin quantification analysis. IR (KBr): $\nu_{\text{N}=\text{N}} \sim 2090 \text{ cm}^{-1}$

■ ASSOCIATED CONTENT

■ Supporting Information

Additional information regarding the characterization of ISA-HCl and diazo-transfer conversions by IR, elemental analysis, ^1H -HRMAS, and HPLC is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

DMF, N,N' -dimethylformamide; DCM, dichloromethane; MeOH, methanol; MeCN, acetonitrile; DMSO, dimethyl sulfoxide; DIEA, diisopropyl ethyl amine; TfN_3 , triflyl azide; ISA-HCl, imidazole-1-sulfonyl azide hydrochloride; AMPS, aminomethyl polystyrene; MBHA, 4-methylbenzhydrylamine polystyrene; AMCM, aminomethyl-ChemMatrix; TG S NH_2 , tentagel S NH_2 resin; ^1H HRMAS, high-resolution magic angle spinning

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