Phenylenediamine catalysis of "click glycosylations" in water: practical and direct access to unprotected neoglycoconjugates†‡

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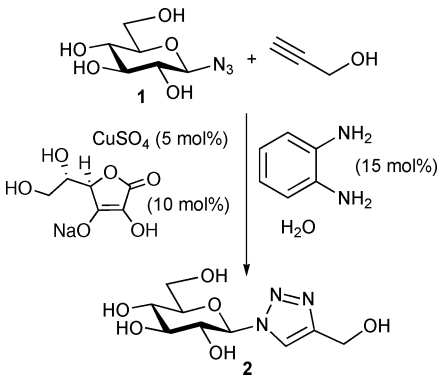
Phenylenediamine-catalyzed click chemistry leads to the efficient, practical, and column-free preparation of neoglycoconjugates from unprotected glucosyl azide, in pure water when aglycon solubility permits.

Carbohydrates and glycoconjugates have been shown to be involved in many cellular recognition events in both normal and pathological processes including cell–cell recognition, host–pathogen or host–symbiont interactions, cancer and metastasis. The unravelling of diversity in glycoconjugate biological functions has been accompanied by the development of synthetic tools allowing the preparation of ever more complex well-defined oligosaccharidic structures. Nevertheless, glycochemistry still requires novel methods and strategies to rapidly access new glycostructures.[1]

Interestingly, recent efforts of many organic chemists focus on a simplification of synthetic procedures towards productivity and diversity.[2] The concept of click chemistry has been introduced[3] in order to mimic the strategies developed by Nature for the modular construction of its major biomolecules (including nucleic acids, proteins, and carbohydrates), and Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC),[4] a regiocontrolled variation of Huisgen's[5] 1,3-dipolar cycloaddtion which has very quickly emerged as the prototype reaction, has found applications in many fields of chemistry.[6] It is important to note that amongst the set of stringent criteria initially introduced as the definition of click chemistry,[3] high yields, simple reaction conditions, readily available reagents and simple non-chromatographic product isolation certainly contributed to the rapid assimilation of the concept by the scientific community.

In this communication we report that when CuAAC between commercially available glucosyl azide **1** and propargyl alcohol is performed with copper sulfate and sodium ascorbate, at room temperature and in pure water,[7] addition of *o*-phenylenediamine leads to completion of the reaction in a much shorter time than expected (less than 45 minutes, compared to more than 4 hours in the absence of *o*-phenylenediamine). This result indicates that *o*-phenylenediamine is accelerating the reaction, possibly *via* the formation of a Cu(I) complex.[8,9] Addition of activated charcoal followed by air oxidation and filtration through Celite then enables non-chromatographic product separation from the catalyst, and isolation of clean triazole **2**.[10]



Scheme 1 Reaction of glucosyl azide **1** with propargyl alcohol, in the presence of catalytic copper sulfate, sodium ascorbate and *o*-phenylenediamine.

In order to illustrate this acceleration effect, we performed a kinetic study by NMR (Fig. 1) which clearly shows that after an initiation time due to the reduction of Cu(II),[11] the conversion rate is significantly accelerated by *o*-phenylenediamine.[12]

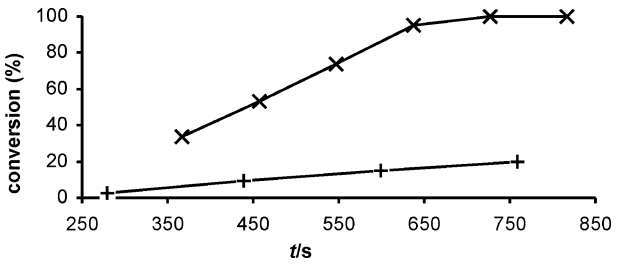


Fig. 1 Kinetic effect of 15.0 mol% (37.5 mM) added *o*-phenylenediamine (×) *versus* no additive (+) on the reaction of β-glucosyl azide (0.25 M) with propargyl alcohol (0.25 M) in the presence of copper sulfate (5.0 mol%, 12.5 mM) and sodium ascorbate (10.0 mol%, 25.0 mM) as determined by ^{1}H-NMR spectroscopy in D_2O under Ar. The conversion was estimated by integration of the H-2 signal of the starting material (δ 3.25 ppm) and anomeric signal of the product (δ 5.75 ppm). The reactions were initiated by copper sulfate addition.

This encouraging result prompted us to apply these conditions to a series of alkynes. Triazole disaccharides **5** and **6** as well as the tris(glucosyl)triazole derivative **7**[13] were obtained in excellent yield (Table 1, entries 4, 5 and 6). This method also allowed efficient access to neoglycosyl amino acid **11** (entry 10) and neoglycolipid **12** (entry 11); in this case, however, some *t*BuOH was added as a co-solvent for aglycon solubility reasons. Alcohols (entries 1

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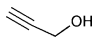
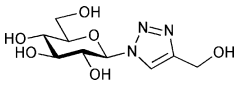
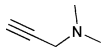
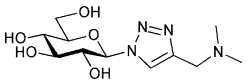
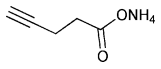
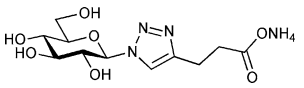
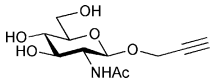
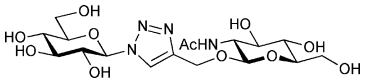
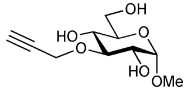
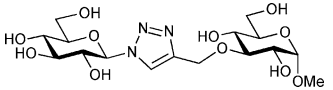
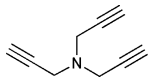
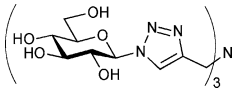
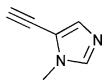
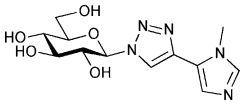
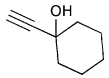
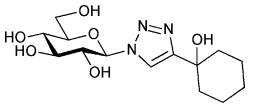
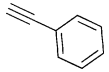
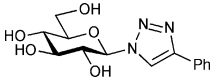
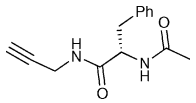
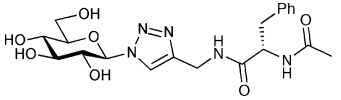
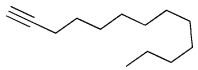
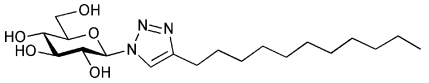
† Electronic supplementary information (ESI) available: Experimental procedures and characterizations, as well as ^{1}H and ^{13}C NMR spectra for compounds **2**–**12**. See DOI: 10.1039/b805528a

‡ Dedicated to Professor Alain Krief on the occasion of his 65th birthday.

Table 1 Phenylenediamine-accelerated reaction of various alkynes with glucosyl azide

Entry	Alkyne	Solvent	Reaction time	Work-up procedure[14]	Product		Isolated yield (%)[a]
1		H_2O	<45 min	A		**2**	98
2		H_2O	<45 min	A		**3**	75
3[b]		H_2O	<2 h	A		**4**	78
4		H_2O	<2 h	A		**5**	95
5		H_2O	<45 min	A		**6**	98
6		H_2O	<45 min	A		**7**	92
7		H_2O–*t*BuOH	<1 h	B		**8**	89
8		H_2O–*t*BuOH	<45 min	B		**9**	98
9		H_2O–*t*BuOH	<45 min	B		**10**	97
10		H_2O–*t*BuOH	<45 min	B		**11**	84
11		H_2O–*t*BuOH	<2 h	C		**12**	85

[a] Yield of product isolated without chromatography. [b] For this reaction, ammonium ascorbate was used instead of sodium ascorbate.

and 8), a tertiary amine (entry 2), a carboxylic acid (entry 3) and aromatics (entries 7 and 9) proved to be compatible with the reaction conditions, and the corresponding neoglycoconjugates were obtained in pure form and very good yields.[14]

We find that of the various strategies that can be used to introduce the required Cu(I) catalyst, the simple reduction of copper sulfate by sodium ascorbate initially suggested by Sharpless[4b] is very convenient; it is also practical and economic, factors that can become crucial on a preparative scale.[15] However, polar unprotected carbohydrate derivatives[16] are intrinsically difficult to isolate from polar byproducts such as copper salts and ascorbic acid oxidation products[17] without chromatography. To solve this problem, we introduce a purification strategy involving the direct trapping of dehydroascorbate (the initial oxidation product of ascorbate) by *o*-phenylenediamine. This addition is known to yield fluorescent quinoxaline derivatives, and has been used for the determination of ascorbate and dehydroascorbate concentrations in biological samples.[18] We show that these derivatives, together with Cu(II),[19,20] can be easily adsorbed onto activated charcoal, allowing their removal by simple filtration. The efficiency of this chromatography-free purification method is exemplified by the ^{1}H-NMR spectrum of crude compound **6** after the final filtration step (Fig. 2).

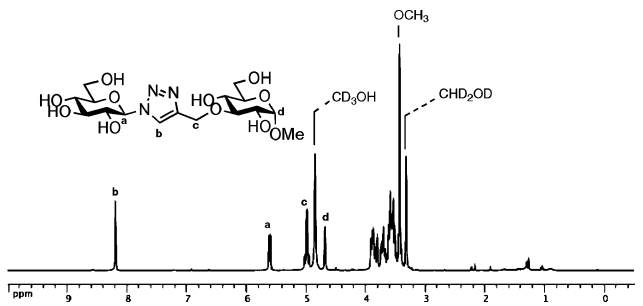


Fig. 2 Full ^{1}H-NMR (CD_3OD, 360 MHz) spectrum of triazole-linked disaccharide **6** after chromatography-free purification.

Significantly, our method proves to be rapid, practical and general, whereas previous reports on the use of click chemistry with unprotected carbohydrates required either heating,[21] microwave activation,[22] copper nanoparticles,[23] or the addition of relatively elaborate copper ligands,[24] in order to avoid prolonged reaction times or an excess of one of the reagents.[25] Furthermore, these methods all relied on chromatographic purification, except in the case of high molecular weight compounds, which allow ultrafiltration.

In conclusion, we believe this strategy can find wide applications in glycoscience, because triazole-linked glycoconjugates can exhibit very interesting biological properties.[26] More generally, other fields should benefit from this method, especially when easy conjugation of polar building blocks is required.

Acknowledgements

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7 An organic cosolvent has commonly been added in previous procedures.

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10 The residual concentration of free copper was estimated using Quantofic® Copper test sticks (Macherey-Nagel) and CHEMets® Kit Copper K-3510 (CHEMetrics) colorimetric assay. Both techniques indicated the removal of 98% of the copper initially introduced (residual copper <0.03 wt%).

11 In the same time, reduction of paramagnetic Cu(II) into diamagnetic Cu(I) is clearly associated with a decrease in line broadening.

12 Studies into the optimization of this effect are currently underway and will be reported in due course.

13 In this case, final copper removal proved more difficult since **7** is probably a good copper ligand. This was easily solved by replacing Celite with neutral alumina in the last filtration step.

14 A typical experimental procedure is given for **6**. To a solution of β-D-glucopyranosyl azide (114 mg, 555 μmol) in H_2O (1560 μL) were added methyl 3-*O*-propargyl-α-D-glucopyranoside (129 mg, 555 μmol, 1 eq.), phenylenediamine (220 μL of a 375 mM solution in H_2O, 8.9 mg, 82.5 μmol, 15 mol%), sodium ascorbate (220 μL of a 250 mM solution in H_2O, 10.9 mg, 55.5 μmol, 10 mol%) and copper sulfate pentahydrate (220 μL of a 125 mM solution in H_2O, 6.9 mg, 27.5 μmol, 5 mol%). The reaction mixture was stirred at room temperature under an argon atmosphere for 45 min, by which time TLC (ethyl acetate–isopropanol–water 3 : 2 : 1) showed complete conversion. Work-up according to Procedure A (below) gave 239 mg (98%) of methyl 3-*O*-[1′-(β-D-glucopyranosyl)-1′*H*-[1′,2′,3′]-triazol-4′-yl]methyl-α-D-glucopyranoside (**6**) as a white solid.
The three work-up procedures are as follows. *Procedure A*: After completion of the reaction, activated charcoal was added to the reaction mixture, which was stirred overnight. The reaction mixture was then filtered through a Celite plug, eluted with water, and the solvent evaporated to dryness to give the expected product. *Procedure B* was followed for reactions with non-water-soluble alkynes (except the more hydrophobic tridec-1-yne): after completion of the reaction, the solvents wereevaporated and the residue was dissolved in MeOH–H_2O (1 : 1), activated charcoal was added and the suspension was stirred overnight. The reaction mixture was filtered through a Celite plug and eluted with MeOH–H_2O (1 : 1). The solvents were evaporated to dryness to give the expected product. *Procedure C* was followed for reaction with tridec-1-yne: after completion of the reaction, activated charcoal was added and the suspension was stirred overnight. The

reaction mixture was then filtered through a Celite plug and washed with *t*BuOH–H_2O (1 : 1). Elution with H_2O–CH_3CN gave the expected product after concentration to dryness.
15 It should also be remembered that most simple Cu(I) salts have poor water solubility.
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26 See for example: M. Touiabia, A. Wellens, T. C. Shiao, Q. Wang, S. Sirois, J. Bouckaert and R. Roy, *ChemMedChem*, 2007, **2**, 1190.