3-Substituted 2-isocyanopyridines as versatile convertible isocyanides for peptidomimetic design

3-Substituted 2-isocyanopyridines as versatile convertible isocyanides for peptidomimetic design

Charlie Hollanders,†[a,b] Mathias Elsocht,†[a] Olivier Van der Poorten,[a] Mouhamad Jida,[a] Evelien Renders,[b] Bert U. W. Maes*[b] and Steven Ballet*[a]

[a] *Research Group of Organic Chemistry, Departments of Chemistry and Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium.*
[b] *Organic Synthesis Division, Department of Chemistry, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium.*
† These authors contributed equally

We report the use of 3-substituted 2-isocyanopyridines as convertible isocyanides in Ugi four-component reactions. The *N*-(3-substituted pyridin-2-yl)amide Ugi products can be cleaved by amines, alcohols, and water with $Zn(OAc)_2$ as catalyst. In addition, the applicability of the method was demonstrated in constrained di-/tripeptides bearing acid and base sensitive protective groups obtained *via* Ugi-4CR post-condensation modifications.

Multicomponent reactions (MCRs) are one-pot transformations with high atom efficiency involving more than two reactants and resulting in single, often structurally diverse, compounds.[1] Due to this diversity, accompanied by an easy-to-use nature, MCRs have become a useful alternative for sequential multistep synthesis and hence, they found considerable application in drug discovery programs.[2-5] The Ugi four-component reactions (Ugi-4CR), in particular, is the method of choice to synthesize dipeptide-like structures. Indeed, this reaction forms an α-acylaminocarboxamide in a single step *via* the condensation of a carbonyl derivative, an amine, a carboxylic acid, and an isocyanide.[6] It has become a powerful tool which, often in combination with post-MCR modifications, is able to generate a wide range of (cyclic) peptide-like structures.[7-9] There is, however, one major drawback related to the Ugi-4CR reaction: the *'C-terminus'* of the condensation product is a very stable secondary amide, derived from the isocyanide component, typically difficult to directly further transform. To allow accessing other functionalities from the amide, convertible isocyanides were developed in the past decades, eventually widening the application of the generated compounds (Figure 1a).[10-14]

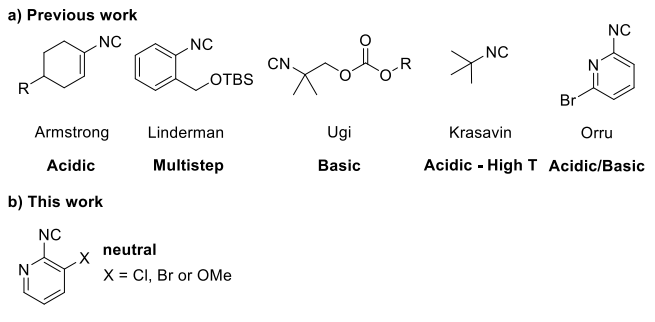
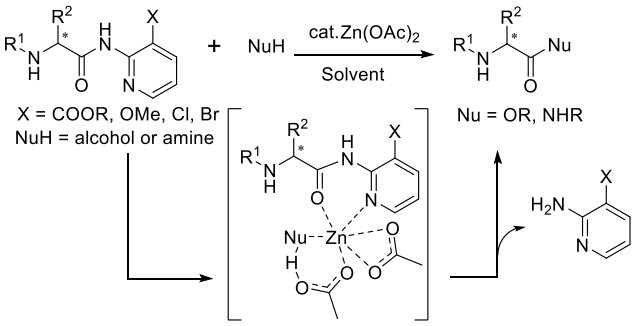


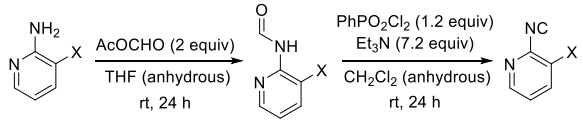
Fig. 1 State of the art convertible isocyanides and their cleavage conditions (a)[10-14] and extension of this methodology to 3-substituted-2-isocyanopyridines (b).

In all cases, an excess of acid or base, and sometimes even multistep transformations employing acidic/basic conditions, is required for the amide cleavage.[10-14] Unfortunately, such conditions are often not compatible with synthetic pathways towards peptidomimetics and potentially present a cumbersome lability for C^{α}-epimerization. In consequence, there remains a high need for convertible isocyanides that can efficiently be cleaved under neutral and mild conditions.



Scheme 1 Directed Zn-catalyzed esterification and transamidation.[15, 16]

Table 1 Synthesis of the 3-substituted 2-isocyanopyridines **3**



1 **2** **3**

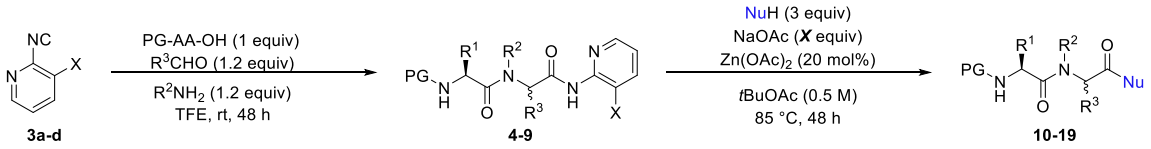
Entry	X	**2**	Yield **2** (%)[a]	X	Yield **3** (%)[b]
1	Br	**2a**	Quant.	**3a**	71
2	Cl	**2b**	Quant.	**3b**	75
3	OMe	**2c**	Quant.	**3c**	74
4	COOMe	**2d**	Quant.	**3d**	55[c]

[a] Isolated yield is reported on 20 mmol scale. [b] Isolated yield on 12 mmol scale is reported. [c] Isolated yield on 6 mmol is reported.

Our laboratories recently developed a Zn-catalyzed alcoholysis[16] and transamidation[15] of primary amides under neutral conditions by introducing a 3-substituted pyridin-2-yl directing group on the primary amide (Scheme 1).[17-20] As halogens,[21] ethers and esters at C3 were previously identified as pyridin-2-yl substituents giving rise to enhanced cleavage kinetics,[16] we reasoned that use of their corresponding isocyanides in the Ugi-4CRs should allow cleavage of the *C*-terminus with nucleophiles under neutral conditions.

The 3-substituted 2-isocyanopyridines **3** were synthesized by *N*-formylation of the 3-substituted 2-amino-pyridine **1** followed by dehydration. Formylation of **1** was achieved in excellent yield using a premade acetic formic anhydride (Table 1). Similar to previously applied procedures[22] and the procedure for 6-bromo-2-isocyanopyridine synthesis,[10] we initially treated the formamide with $POCl_3$. Even with a large excess of $POCl_3$ (up to 2.3 equiv), isocyanides **3a-c** could only be isolated in a very low yield (see ESI, Table S1). In case of the C3-ester substituent, full degradation was observed. Interestingly, $PhPO_2Cl_2$ proved to be a valid alternative,[23] as this dehydrating agent smoothly gave access to the four isocyanides of type **3** with a significant improvement in yield (Table 1). Subsequently, the isocyanides **3** were evaluated in the Ugi-4CR reaction with propylamine, isovaleraldehyde and Boc-L-Phe-OH. Initial evaluation of **3a-c** using a 1:1:1:1 ratio of the components in trifluoroethanol (TFE) at room temperature showed approximately 70% HPLC conversion to the Ugi products **4a-c** after 48 hours (See ESI, Table S2). Use of the C3-ester isocyanide **3d**, however, gave unidentified side products and degradation of the isocyanide, most likely due to a lack of compatibility with TFE. This urged us to further optimise the reaction (See ESI, Table S3, Table S4, and Table S5) for this particular isocyanide. Here, the best result was obtained using CH_2Cl_2 in combination with microwave (μW) heating at 110 °C for 30 minutes, giving an isolated yield of 41% (See ESI, Scheme S2). Unfortunately, these conditions were not transferable upon application of other amines, aldehydes or carboxylic acids in this Ugi reaction, and therefore isocyanide **3d** was nolonger considered. Further efforts to improve the reaction with isocyanides **3a-c** (See ESI, Table S6-8) yielded appropriate conditions allowing isolation of **4a-c** with yields ranging from 66-77% (Table 2, Step 1). The broader applicability of these conditions was verified by changing the aldehyde, amine, and *N*-Boc protected amino acid components, providing the different Ugi products **5-8** in moderate to good yields (Table 2 and See ESI, Scheme S2). The scope was extended towards *N*-Fmoc protected amino acid (Table 2, Entry 15) by the synthesis of **9** that was isolated in a good yield with 1.5 equiv of isocyanide **3b**. Next, the three different pyridine directing groups featuring a Br, Cl or OMe were evaluated in Zn-catalyzed transamidation.[15, 24-26] Starting from our previously published conditions,[15] 3 equivalents of H-L-Phe-OMe.HCl and NaOAc as a base in THF at 70 °C for 24 h were utilized, providing NMR yields of 44-47% (See ESI, Table S9).

Table 2 Use of the isocyanides **3a-d** in the Ugi-4CR and amide cleavage.



3a-d **4-9** **10-19**

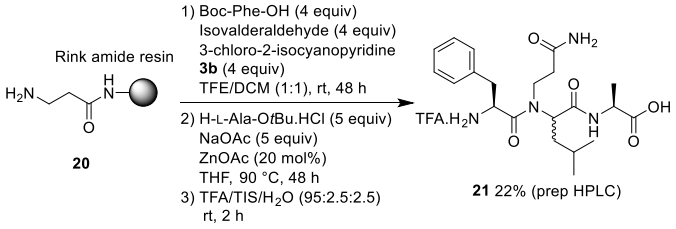
	Step 1						Step 2			
Entry	PG-AA-OH	R^2	R^3	X	**4-8**	Yield (%)[a]	NuH	NaOAc (***X*** equiv)	**10-19**	Yield (%)[e]
1	Boc-Phe	Pr	*i*Bu	Br	**4a**	68	H-Phe-OMe.HCl	3	**10**	60 (85)
2	Boc-Phe	Pr	*i*Bu	Cl	**4b**	77	H-Phe-OMe.HCl	3	**10**	85 (91)
3	Boc-Phe	Pr	*i*Bu	OMe	**4c**	66	H-Phe-OMe.HCl	3	**10**	68 (90)
4	Boc-Ser(O*t*Bu)	Pr	*i*Bu	Cl	**5b**	73	H-Phe-OMe.HCl	3	**11**	72 (78)
5	Boc-Trp	Bn	*i*Bu	Cl	**6b**	66	H-Phe-OMe.HCl	3	**12**	77 (100)
6	Boc-Val	Bn	(4-Br)-Ph	Cl	**7b**	48	H-Phe-OMe.HCl	3	**13**	72 (89)
7	Boc-Asp(OAll)	(4-Cl)-$PhCH_2CH_2$	Me	Cl	**8**	48	H-Phe-OMe.HCl	3	**14**	61 (81)
8	Fmoc-Phe	Cyclopropyl	H	Cl	**9**	46[c,d]	H-Phe-OMe.HCl	3	**15**	73 (85)
9	Boc-Phe	Pr	*i*Bu	Cl	**4b**		H-Val-O*t*Bu.HCl	3	**16**	80 (100)
10	Boc-Phe	Pr	*i*Bu	Cl	**4b**		H-Lys(Cbz)-OMe.HCl	3	**17**	55 (80)
11	Boc-Phe	Pr	*i*Bu	Cl	**4b**		H_2O	0	**18**	94 (100)
12	Boc-Phe	Pr	*i*Bu	Cl	**4b**		MeOH	0	**19**	81 (100)

[a] Isolated yield with a 1:1 diastereomeric ratio on 1 mmol scale is reported. [b] CH_2Cl_2 as solvent at 110 °C in μW for 0.5 h. [c] 3-chloro-2-isocyanopyridine **3b** (1.5 equiv). [d] Isolated yield as a single enantiomer. [e] Isolated yield with a 1:1 diastereomeric ratio on 0.25 mmol scale is reported, ^{1}H NMR yield determined with internal standard between brackets.

Optimization of the transformation on Ugi product **4b** (See ESI, Table S10) showed that by changing the solvent to *t*BuOAc and increasing the temperature to 85 °C for 48 hours, an excellent NMR yield of 91% and an isolated yield of 85% of **10** was achieved. Applying these conditions on **4a** (X = Br) and **4c** (X = OMe) also showed high NMR yield but lower isolated yields (**10** in 60% and 68% yield, respectively) (See ESI, Table S12 and Scheme S3). The 3-chloropyridin-2-yl directing group was therefore retained as optimal. As there is a significant structural similarity with the 6-bromo-2-isocyanopyridine previously published by Orru and co-workers[10], the Zn-catalyzed transamidation of a 6-Br pyridin-2-yl-bearing amide (for the synthesis see ESI, Scheme S1) was examined. Remarkably, no cleavage of the amide was observed, illustrating the importance of the substituent's position on the pyridin-2-yl directing group (3-Br in **4a** versus 6-Br). This is presumably due to the blocking of the Zn coordination as it is a C2,C6 disubstituted pyridine.

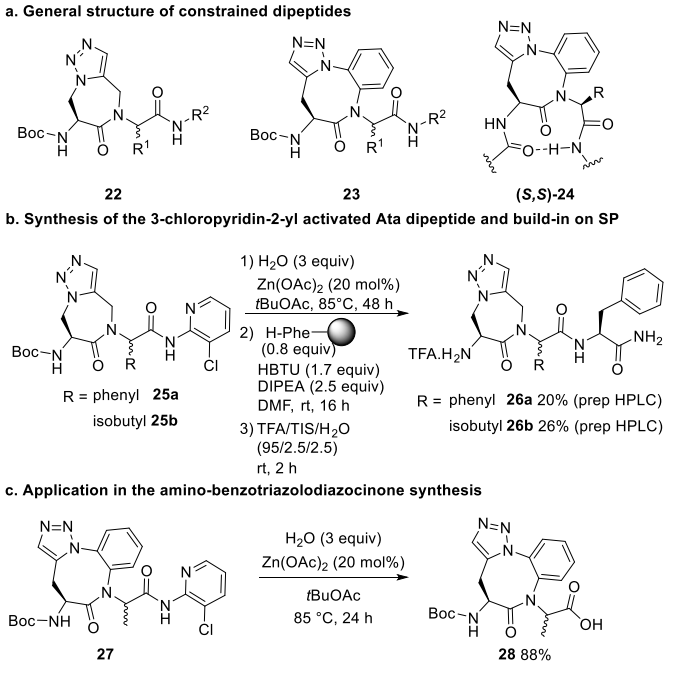
To demonstrate the transformation's feasibility on other Ugi substrates, the Cl-bearing Ugi products **5b**, **6b**, **7b**, **8** and **9** were used in combination with H-L-Phe-OMe.HCl as the nucleophile. In all cases, a good conversion was observed, allowing the isolation of **10-15** in good yields. Particularly interesting is **8** and **9** featuring an *O*-allyl ester or *N*-Fmoc group, respectively, representing protective functionalities often applied in peptide chemistry. The scope of the nucleophile component was extended to the formation of **16** and **17**, which demonstrated a

compatibility with β-branched and *N*-Cbz protected amino acids, respectively. Additionally, hydrolysis and methanolysis of Ugi product **4b** was achieved to provide carboxylic acid **18** and methyl ester **19**, respectively. Both Zn-catalyzed reactions went to full reaction conversion in the presence of 3 equivalents of H_2O or MeOH, allowing their isolation in excellent yields (Table 2, Entry 11 and 12). The reaction with water is particularly interesting as it is the first example employing water as a nucleophile giving access to carboxylic acids from amides under neutral reaction conditions. To the best of our knowledge there are also no examples of such reactions with other amide activating strategies.



Scheme 2 On-resin Ugi reaction and transamidation with reported, combined isolated yield for the separated diastereomers.

Subsequently, the methodology's compatibility with solid-phase organic synthesis (SPOS) was demonstrated by the preparation of **21**. Herein, the Ugi reaction was performed with a Rink amide resin-bound β-Ala residue in TFE/DCM. This solvent mixture was applied to allow proper swelling of the resin. After 48 h, the reaction solution was removed by filtration and the resin was subjected to our previously reported transamidation conditions on solid-support.[15] Following TFA/TIS/H_2O cleavage from the resin, the two diastereomers of the tripeptide **21** were separated using preparative HPLC.



Scheme 3 General structure of Ata **22** and amino-benzotriazolodiazocinone **23**, and observed β-turn with hydrogen bond (dotted line) in (*S,S*)-**24**.(a) Application of amide cleavage in the Ata-scaffold **25** (for synthesis is ESI, Scheme S4) in SPPS with reported, combined isolated yield for the separated diastereomers. (b) Application of hydrolysis in the amino-benzotriazolodiazocinone **27** (for synthesis see ESI, Scheme S5) **. (c)**

Moreover, the methodology's versatility was established through the synthesis of two previously published constrained dipeptide, which were obtained by Ugi-post-condensation modifications.[22, 27] The aminotriazoloazepinone (Ata) scaffold **22** (Scheme 3a) was previously obtained *via* a multi-step synthesis,[28] and recently accessed through a one-pot Ugi-Huisgen reaction.[22] In the latter, the cycloaddition was performed by heating the Ugi reaction mixture to 70 °C to obtain the desired Ata scaffold. Nonetheless, the *C*-terminal amide restricts the application of building blocks of type **22** towards more elaborated peptidic compounds, and hence this could potentially be overcome with the convertible isocyanides described herein. When using convertible isocyanide **3b**, heating of Ugi product in TFE gave the corresponding ester in rather low yield (See ESI, Scheme S4). Besides cycloaddition, a non-catalytic, undesired alcoholysis occurred. Fluorinated alcohols are known to be more acidic and strong hydrogen bonding donors rationalizing this observation.[29]

Alternatively, to obtain the target Ata scaffolds **25** (See ESI, Scheme S4), a solvent switch was performed prior to heating in the one-pot reaction, allowing isolation of **25** in decent yields, considering the structural complexity of the reaction product. The use of **25** as a precursor for solid phase peptide synthesis (SPPS) was investigated. To uncage the (3-chloropyridinyl)amide in **25**, hydrolysis in the presence of water and $Zn(OAc)_2$ catalyst was performed at 85 °C in *t*BuOAc (Scheme 3b). Full conversion to the carboxylic acid allowed subsequent coupling of the dipeptide to a solid phase anchored phenylalanine without intermediate purification. The Ata-bearing tripeptide **26** was cleaved from the resin and isolated using preparative HPLC, as a proof-of-principle. More extended sequences encompassing the Ata-building block are hence within reach thanks to the developed methodology.

As a second example, we applied the catalyst-free one-pot Ugi-Huisgen sequence to obtain constrained eight-membered dipeptide analogues of type **23** (Scheme 3a).[27] Such a template is highly relevant in peptidomimetic design, as *in silico* molecular modelling and NMR studies demonstrated the turn-inducing properties. More specifically, the lowest energy conformations of (*S,S*)-**24** adopt a β-turn conformation stabilized with hydrogen bond (dotted line in **24**).[27] Accordingly, Ugi reaction of 3-chloro-2-isocyanopyridine **3b**, acetaldehyde, propargylglycine and 2-azidoaniline in TFE at room temperature for 24 h provides full conversion of the azide. To avoid alcoholysis of the (3-chloropyridinyl)amide (*vide supra*, Scheme S6), the solvent was switched to 1,4-dioxane and the mixture was subsequently heated to 100 °C allowing selective Huisgen cycloaddition. These conditions were ideally suited to give an 8-membered ring **27**. Amide hydrolysis into the corresponding carboxylic acid **28** was performed in high yield under standard conditions, allowing further amino acid coupling *via* SPPS.

In summary, 3-substituted 2-isocyanopyridines are introduced as novel convertible isocyanides. The current study showcases facile amide convertibility of the Ugi-4CR products synthesized employing these isocyanides under mild and neutral reaction conditions with nucleophiles. No base or acid was required, only catalytic $Zn(OAc)_2$. Transamidation, alcoholysis as well as hydrolysis can be smoothly performed. Reactions with water are to the best of our knowledge unprecedented in cleavage reactions with activated amides. The practical utility in synthesis was successfully demonstrated through the preparation of two conformationally constrained dipeptidomimetic scaffolds. Given the versatility and applicability of this methodology, it is expected that these convertible 3-substituted 2-isocyanopyridines will be preferred isocyanide reagents in multicomponent reactions for advanced peptidomimetic design.

This work was financially supported by the Research Foundation Flanders (FWO), the Francqui Foundation and BOF UA. SB also acknowledges the Strategic Research Program (SRP) of the Vrije Universiteit Brussel for financial support.

Conflicts of interest

There are no conflicts to declare.

Author contributions

C.H., M.E., O.V.D.P., M.J. and E.R. carried out the experiments and characterized all synthesized compounds. B.U.W.M. and S.B. designed the experiments and coordinated the study. All authors contributed to the writing of the manuscript.

Notes and references

1 A. Dömling, *Chem. Rev.*, 2006, **106**, 17-89.
2 I. Akritopoulou-Zanze, *Curr. Opin. Chem. Biol.*, 2008, **12**, 324-331.
3 A. Domling, W. Wang and K. Wang, *Chem. Rev.*, 2012, **112**, 3083-3135.
4 P. Slobbe, E. Ruijter and R. V. A. Orru, *MedChemComm*, 2012, **3**, 1189-1218.
5 E. Ruijter and R. V. A. Orru, *Drug Discov. Today Technol.*, 2013, **10**, e15-e20.
6 M. A. Fouad, H. Abdel-Hamid and M. S. Ayoup, *RSC Advances*, 2020, **10**, 42644-42681.
7 G. Koopmanschap, E. Ruijter and R. V. Orru, *Beilstein J. Org. Chem.*, 2014, **10**, 544-598.
8 S. Zaretsky and A. K. Yudin, *Drug Discov. Today Technol.*, 2017, **26**, 3-10.
9 L. Reguera and D. G. Rivera, *Chem. Rev.*, 2019, **119**, 9836-9860.
10 G. van der Heijden, J. A. W. Jong, E. Ruijter and R. V. A. Orru, *Org. Lett.*, 2016, **18**, 984-987.
11 R. J. Linderman, S. Binet and S. R. Petrich, *J. Org. Chem.*, 1999, **64**, 336-337.
12 T. Lindhorst, H. Bock and I. Ugi, *Tetrahedron*, 1999, **55**, 7411-7420.
13 S. Tsirulnikov, M. Nikulnikov, V. Kysil, A. Ivachtchenko and M. Krasavin, *Tetrahedron Lett.*, 2009, **50**, 5529-5531.
14 T. A. Keating and R. W. Armstrong, *J. Am. Chem. Soc.*, 1995, **117**, 7842-7843.
15 K. Hollanders, E. Renders, C. Gadais, D. Masullo, L. Van Reamdonck, C. C. D. Wybon, C. Martin, W. A. Herrebout, B. U. W. Maes and S. Ballet, *ACS Catal.*, 2020, **10**, 4280-4289.
16 C. C. D. Wybon, C. Mensch, K. Hollanders, C. Gadais, W. A. Herrebout, S. Ballet and B. U. W. Maes, *ACS Catal.*, 2018, **8**, 203-218.
17 D. Kaiser, A. Bauer, M. Lemmerer and N. Maulide, *Chem. Soc. Rev.*, 2018, **47**, 7899-7925.
18 M. B. Chaudhari and B. Gnanaprakasam, *Chem.: Asian J*, 2019, **14**, 76-93.
19 J. Buchspies and M. Szostak, *Catalysts*, 2019, **9**, 53, https://doi.org/10.3390/catal9010053
20 Y. Bourne-Branchu, C. Gosmini and G. Danoun, *Chem. Eur. J.*, 2019, **25**, 2663-2674.
21 The C3 Bromo was incorporated as a direct comparison with the 6-bromopyridin-2-yl group introduced by the Orru group.
22 T. M. A. Barlow, M. Jida, D. Tourwé and S. Ballet, *Org. Biomol. Chem.*, 2014, **12**, 6986-6989.
23 G. Kobayashi, T. Saito and Y. Kitano, *Synthesis*, 2011, 3225-3234.
24 J. E. Dander, E. L. Baker and N. K. Garg, *Chem. Sci.*, 2017, **8**, 6433-6438.
25 T. Zhou, G. Li, S. P. Nolan and M. Szostak, *Org. Lett.*, 2019, **21**, 3304-3309.
26 G. Li and M. Szostak, *Nat. Commun.*, 2018, **9**, 4165.
27 T. M. A. Barlow, M. Jida, K. Guillemyn, D. Tourwé, V. Caveliers and S. Ballet, *Org. Biomol. Chem.*, 2016, **14**, 4669-4677.
28 K. Buysse, J. Farard, A. Nikolaou, P. Vanderheyden, G. Vauquelin, D. Sejer Pedersen, D. Tourwé and S. Ballet, *Org. Lett.*, 2011, **13**, 6468-6471.
29 X.-D. An and J. Xiao, *Chem.*, 2020, **20**, 142-161.