Rapid construction of substituted 3-amino-1,5-benzothiazepin-4(5H)-one dipeptide scaffolds through an Ugi-4CR-Ullmann cross-coupling sequence

Rapid Construction of Substituted 3-Amino-1,5-Benzothiazepin-4(5*H*)-One Dipeptide Scaffolds Through an Ugi-4CR – Ullmann Cross-Coupling Sequence

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A 3-step methodology for the synthesis of 1,5-benzothiazepin-4(5*H*)-one dipeptidomimetics has been elaborated via an Ugi-4CR followed by a *S*-trityl deprotection and an intramolecular Cu(I)-catalyzed Ullmann condensation with moderate to good yields. *In silico* and NMR conformational studies showed that the lowest energy conformers stabilize γ- and β-turn structures.

Substituted benzothiazepines and benzothiazepinones have a prominent place in the realm of bioactive compounds,[1, 2] with a therapeutic scope including anticancer agents,[3] calcium channel blockers and antihypertensive agents,[4, 5] angiotensin converting enzyme inhibitors,[6] human leukocyte elastase inhibitors,[7] and bradykinin receptor agonists.[8, 9] The first 1,5-benzothiazepin-4(5*H*)-one-based therapeutics included the cardiovascular agents Diltiazem **1** and Clentiazem **2**, and Thiazesim **3** as an antidepressant (Fig. 1). Upon substitution of the Pro[7]-Phe[8] segment in bradykinin and D-Tic[7]-Oic[8] in HOE140 with the (*S*)-1,5-benzothiazepin-4(5*H*)-one dipeptide mimetic core (D-BT, indicated in red in **4**), full potent and selective bradykinin B_2 receptor agonists (of type **4**) were discovered and a type II' β-turn in the *C*-terminal tetrapeptide segment of the [D-BT]bradykinin analogues was observed (Fig. 1).[8, 9] This result was in line with the report that the D-BT motif induced a type II' β-turn in both solution and solid state.[10] Additionally, potent and highly selective bradykinin B_1 receptor antagonists were obtained via the introduction of the D-BT dipeptide scaffold in [des-Arg]bradykinin analogues.[11, 12]

In order to access the D-BT scaffold, Amblard and co-workers successfully adapted the original procedure described by Slade[6] starting from the condensation of Boc-D-Cys-OH and *o*-fluoronitrobenzene into **5**, followed by nitro reduction and intramolecular lactamization to provide **6**.[13] Subsequent alkylation of the lactam nitrogen with methyl bromoacetate yielded 1,5-benzothiazepin-4(5*H*)-one dipeptidomimetic **7** (Scheme 1). However, when aiming for 1,5-benzothiazepinone-based dipeptidomimetics **8** including substituents at the C_α of the exocyclic amino acid, *N*-alkylation is problematic and synthetic protocols to introduce substituents at the C_α-center in **7** proved difficult.

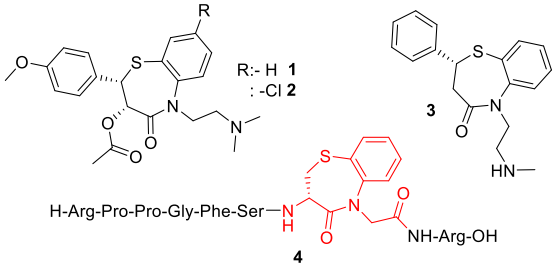
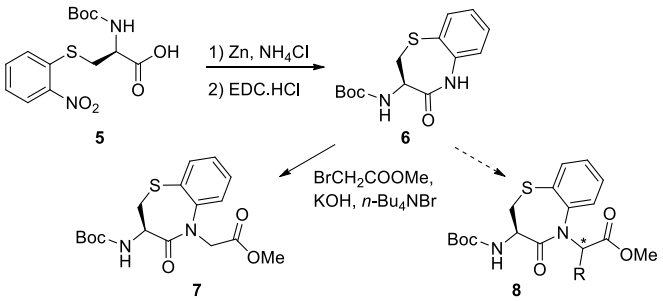
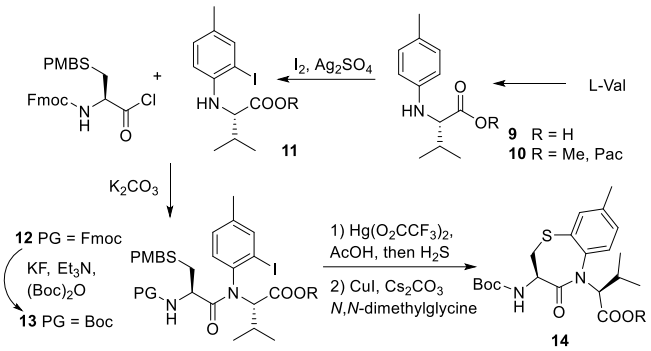


Fig. 1 Selected examples of 1,5-benzothiazepin-4(5*H*)-one-based pharmaceuticals **1**-**3** and a potent bradykinin B_2 receptor agonist **4**.



Scheme 1 Synthesis of D-BT **7**[6, 13] and problematic extension towards C_α-substituted analogues **8**.

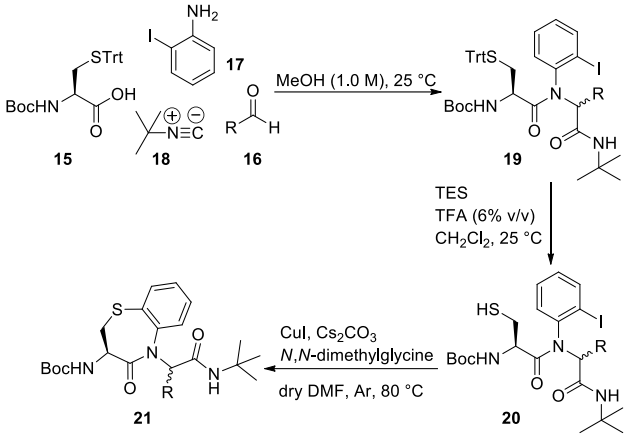
For this reason, Gan and Ma reported an alternative route in which several 1,5-benzothiazepinone dipeptide mimics were generated through two CuI-catalyzed cross coupling reactions (Scheme 2).[14] *N*-tolyl-L-Val-OH **9** was first obtained via a CuI-catalyzed coupling of *p*-bromotoluene with L-Val. Methyl or phenacyl (Pac) ester **10** were then *o*-iodinated to give **11**. After condensation of **11** with Fmoc-L-Cys(PMB)-Cl (PMB: *p*-methoxybenzyl) and switching the Fmoc protecting group in **12** to a Boc group, the *S*-PMB group in **13** was removed and the free thiol was subjected to an intramolecular CuI/*N*,*N*-dimethylglycine catalyzed C-S Ullmann condensation to give the Boc-protected (*R,S*)-1,5-benzothiazepin-4(5*H*)-one dipeptide mimetic **14**. This methodology allowed the introduction of various N^5-substituents by changing the amino acid coupling partners (i.e. L-Ala, L-Val, L-Ile, L-Phe), but it had the intrinsic limitation of requiring a *p*-methyl group in **10**, in order to promote *o*-selective iodination. The overall yield of this six step procedure was 9 -17%.[14]



Scheme 2 Synthesis of 1,5-benzothiazepin-4(5*H*)-one dipeptidomimetics **14** via two CuI-catalyzed cross couplings.[14]

In this work, a novel short synthetic strategy was developed which comprised an Ugi-4CR[15, 16] and an intramolecular Cu(I)-catalyzed C-S cyclization reaction as the two key synthetic steps (Scheme 3). Moreover, our goal was to generate a diverse set of substituted 1,5-benzothiazepin-4(5*H*)-one-based dipeptide mimetics, wherein the C_α-position of the exocyclic amino acid was substituted with different alkyl and aryl substituents. Starting from commercially available Boc-L-Cys(Trt)-OH **15**, different aldehydes **16**, *o*-iodoaniline **17**, and *tert*-butylisocyanide **18**, linear Ugi dipeptides of type **19** were constructed. Orthogonal *S*-trityl deprotection into **20** was followed by a CuI-catalyzed C-S Ullmann condensation, yielding the desired (*R*)-1,5-benzothiazepin-4(5*H*)-one-containing dipeptide mimetics **21**. Using various aliphatic and aromatic aldehydes **16**, seven linear Ugi-4CR dipeptides **19a-g** were obtained (Table 1). For reasons of product solubility and a reported superior reaction conversion, the Ugi-4CR reactions were run in methanol rather than in the commonly employed 2,2,2-trifluoroethanol.[17, 18] During optimization of the Ugi-4CR reaction, increased reaction temperatures resulted in substantial formation of side products, yielding complex crude reaction mixtures.

To improve reaction conversions, imine preformation was attempted prior to addition of Boc-L-Cys(Trt)-OH **15** and *tert*-butylisocyanide **18**, but this did not prove to be effective. Nonetheless, rewarding isolated yields between 44 and 88% of Ugi-4CR diastereomers **19a-g** were obtained when mixing the four components **15-18** at once at room temperature (Table 1). Diastereomeric ratios (dr) of **19a-f** were determined to be 1:1 via RP-HPLC analysis, except for **19g** (dr 2:1), where isobutyraldehyde was used as an α-branched aliphatic aldehyde.[19] Purification of the crude Ugi-4CR products **19a-g** via silica gel column chromatography proved necessary to allow clean subsequent *S*-trityl deprotection towards **20a-g**. This deprotection was carried out in a CH_2Cl_2/TFA mixture (94:6 v/v), in presence of an excess of triethylsilane as trityl cation



Scheme 3 Optimized synthetic pathway towards (*R*)-1,5-benzothiazepin-4(5*H*)-one dipeptide mimics **21** via subsequent Ugi-4CR, *S*-trityl deprotection and CuI-catalyzed C-S Ullmann condensation.

scavenger. The reaction was closely monitored via RP-HPLC analysis and proved in all cases to be completed after 15 minutes. Subsequent silica gel column chromatography allowed to isolate *S*-trityl deprotected Ugi-4CR products **20a-g** in good yields (Table 1). In a next step, formation of the aryl-sulfur bond was first attempted on substrate **20a** by subjecting the unprotected thiol to an intramolecular metal-catalyzed *S*-arylation through use of the aryl iodide. Different reaction conditions were screened ranging from CuI/ethane-1,2-diol/K_2CO_3,[20] CuI/2,9-dimethyl-1,10-phenanthroline/sodium *tert*-butoxide,[21] and Pd_2dba_3-$CHCl_3$/dppf/Et_3N.[22] Although LC-MS and ^{13}C NMR analysis indicated formation of the targeted dipeptidomimetic **21a** for all tested conditions, reaction mixtures with unidentified side products were obtained. However, a clean conversion into **21a** was noticed upon application of CuI/*N*,*N*-dimethylglycine/Cs_2CO_3 in dried and degassed DMF at 80 °C under argon atmosphere.[14] These reaction conditions were successfully applied for all other scaffolds **21b-g** (Table 1). All *S*-trityl deprotected Ugi-4CR substrates **20a-g** were completely consumed within a time frame of 3-4 hours. Purification via silica gel column chromatography afforded the 1,5-benzothiazepin-4(5*H*)-ones **21a-g** as a mixture of diastereomers in moderate to good yields (Table 1). It should be noted that for all cases **21a-g**, both diastereomers were separable via column chromatography, yet moderate yields were sometimes obtained due to partial co-elution. As observed via RP-HPLC and ^1H NMR analysis, the diastereomeric ratios obtained after CuI-catalyzed intramolecular cyclization deviated from those in the precursors **20** (Table 1). To rule out epimerization by base-induced enolization, diastereomers of **20b** were separately mixed with an excess of Cs_2CO_3 in DMF at 80°C. After 1 hour of reaction no epimerization was observed. In a next step, the diastereomers of **20b** were separately subjected to the C-S Ullmann coupling to detect any energetically favored formation of one diastereomer of **21b**. These experiments revealed that several unidentified side products were formed during the CuI-catalyzed cyclization into (*R*,*R*)-**21b**, whereas reaction of (*R*,*S*)-**20b** resulted in a clean conversion into (*R*,*S*)-**21b** (see ESI).

Table 1 Linear Ugi-4CR products **19a-g**, *S*-trityl deprotected Ugi-4CR products **20a-g**, and 1,5-benzothiazepin-4(5*H*)-one dipeptides **21a-g** with their respective isolated yields and diastereomeric ratios.

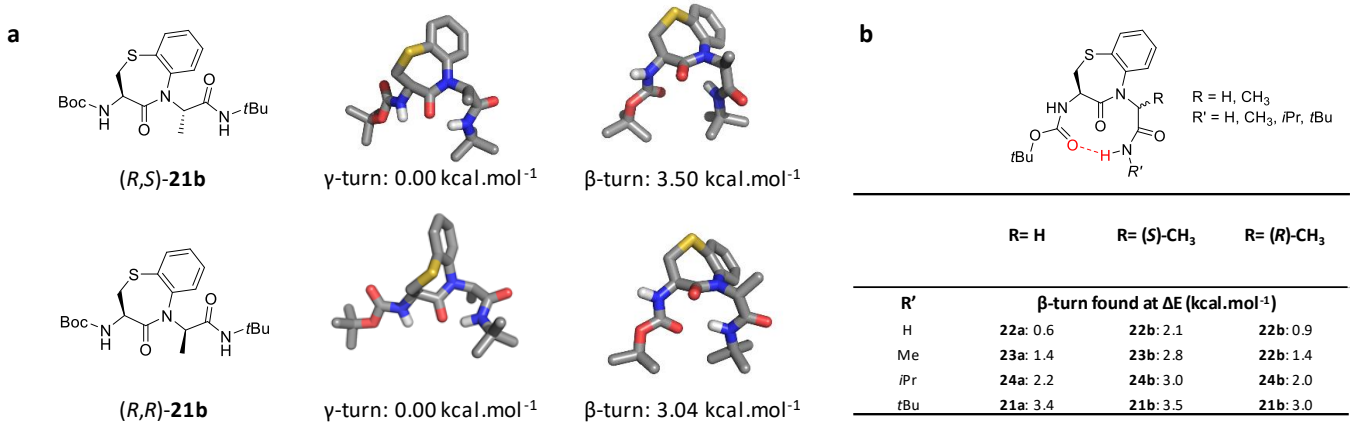
Aldehyde (R-CHO)		**Ugi-4CR product 19**		***S*-trityl deprotected 20**		**1,5-benzothiazepinone 21**	
Structure	**Entry**	**Yield**[a]	**dr**[b]	**Yield**[a]	**dr**[b]	**Yield**[a]	**dr**[b]
H-CHO	**a**	84	/	82	/	78	/
CH_3-CHO	**b**	88	1:1	79	1:1	72	3:4
	**c**	80	1:1	73	1:1	46	1:4
	**d**	51	1:1	64	1:1	61	2:3
	**e**	46	1:1	68	1:1	51	1:4
	**f**	44	1:1	72	1:1	49	1:5
	**g**	52	2:1	63	1:2	31	3:7

[a] Isolated yields obtained after silica gel column chromatography. [b] dr was determined by integration of the corresponding RP-HPLC peak areas according to their increasing retention times.

The effect of the second stereocenter on the overall turn-inducing properties of the 1,5-benzothiazepin-4(5*H*)-one dipeptidomimetics **21a-g** was explored via structural *in silico* calculations. Molecular modeling indicated that all lowest energy conformers of (*R*,*S*)- and (*R*,*R*)-benzothiazepinones **21a-g** possessed a hydrogen bond-stabilized γ-turn conformation (Fig. 2a). However, the expected β-turn conformations were also observed within a range of 1.95 to 5.60 kcal.mol^{-1} above the energetically lowest conformer (ESI†). The conformational preferences and overall turn-inducing properties of dipeptidomimetics **21a-g** can potentially be influenced by the steric bulk of the *C*-terminal *tert*-butylamide moiety. Therefore, structural calculations were repeated for **21a** and both (*R*,*S*)- and (*R*,*R*)-**21b** with less sterically hindering primary and secondary amides (i.e. -$CONH_2$ **22a/b**, -CONHMe **23a/b**, -CONH*i*Pr **24a/b**) (Fig. 2b). Indeed, significantly lower energy differences were recorded between the lowest energy γ-turn conformers and the first β-turn structures if the *C*-terminal *tert*-butylamide group was replaced. The calculations also showed the direct influence of the exocyclic C_α-stereocenter on the overall turn properties of (*R*,*S*)- and (*R*,*R*)-diastereomers of **21a-g**. In case of the (*R*,*R*)-stereochemistry, the first reported β-turn structures were observed at significantly lower energy differences with respect to the minimum energy structure, as compared to their (*R*,*S*)-analogues, and these ΔE values were even more pronounced with less sterically hindering *C*-terminal amide substituents (Fig 2b + ESI).

To probe these conformational findings experimentally, both (*R*,*S*)- and (*R*,*R*)-diastereomers of **21b** were separated by chromatography and conformational preferences were further studied via ^{1}H NMR analysis. First, the individual stereochemistry of both (*R*,*S*)- and (*R*,*R*)-**21b** was assigned via 2D ^{1}H NOESY studies (ESI). For (*R*,*R*)-**21b**, an indicative NOE correlation was observed between the CH_3^β group of the '(*R*)-Ala residue' and the H-6 of the aromatic benzothiazepinone ring (a distance of 2.8 Å was found in the lowest energy conformer by MM) (ESI). This distinct NOE signal was absent for (*R*,*S*)-**21b** (MM 5.0 Å in the lowest energy conformer) (ESI). Next, ^{1}H NMR spectra of (*R*,*S*)- and (*R*,*R*)-**21b** were measured in $CDCl_3$ and DMSO-d_6 to evaluate the effect of switching from a non-hydrogen bond-forming solvent to a strong hydrogen bond-forming solvent on the chemical shift value of the *N*H-*tert*-butylamide proton. Both amide resonances of (*R*,*S*)- and (*R*,*R*)-**21b** were only weakly influenced upon the

solvent switch, resulting in small differences in chemical shift values of, respectively, 0.35 and 0.41 ppm, indicating that the *N*H-amide proton is solvent-shielded.



	R= H	R= (*S*)-CH$_3$	R= (*R*)-CH$_3$
R'	β-turn found at ΔE (kcal.mol^{-1})		
H	**22a**: 0.6	**22b**: 2.1	**22b**: 0.9
Me	**23a**: 1.4	**23b**: 2.8	**22b**: 1.4
*i*Pr	**24a**: 2.2	**24b**: 3.0	**24b**: 2.0
*t*Bu	**21a**: 3.4	**21b**: 3.5	**21b**: 3.0

Fig. 2 Lowest energy β-turn conformations of (*R*,*S*)- and (*R*,*R*)-**21b**, relative to their lowest energy γ-turn conformations (0.00 kcal.mol^{-1}) as a reference (a); Energy differences (kcal.mol^{-1}), relative to the lowest energy γ-turn conformation (0.00 kcal.mol^{-1}) as a reference , for the first β-turn structures of **21a-24a** and (*R*,*S*)- and (*R*,*R*)-**21b-24b** as a function of various *C*-terminal amide group substituents (b).

Unlike intermolecularly hydrogen bonded amide protons, intramolecularly hydrogen bonded ones are only slightly affected by the temperature of the system.[23, 24] Samples of (*R*,*S*)- and (*R*,*R*)-**21b** were then heated in DMSO-d_6 (temperature range between 298 K and 348 K, with temperature increments of 5 K). Only coefficients between 0 and -4 ppb/K are generally accepted for solvent-shielded amide protons involved in intramolecular hydrogen bonds. Given the fact that this criterion has been set for cyclic peptides or peptides with longer sequences, in small peptides a thermal coefficient less negative than -4.6 ppb/K has been suggested as a criterion for identifying the solvent-shielded amide protons.[23] For (*R*,*S*)-and (*R*,*R*)-**21b**, the chemical shift resonances of the *N*H-*tert*-butylamide proton shifted linearly, resulting in temperature coefficients of, respectively, -3.6 and -2.2 ppb/K. Hence, the calculated amide proton temperature coefficients for (*R*,*S*)- and (*R*,*R*)-**21b** were supporting the presence of an intramolecular hydrogen bond, a common characteristic of γ- and β-turns. These experimental results were consistent with the molecular modeling of both diastereomers of mimic **21b**.

In conclusion, an efficient and rapid synthesis of substituted 1,5-benzothiazepin-4(5*H*)-one dipeptide mimics is described. These scaffolds were constructed via an Ugi-4CR followed by an intramolecular Cu(I)-catalyzed Ullmann condensation, presenting 1,5-benzothiazepin-4(5*H*)-ones with moderate to good overall yields using simple experimental procedures. Through this strategy, access is provided to dipeptidomimetics containing both diastereomers of the exocyclic α-substituted amino acid. After separation of the diastereomers, we demonstrated that both (*R*,*S*)- and (*R*,*R*)-diastereomers adopt turn conformations stabilized by an intramolecular hydrogen bond. Ongoing efforts are focused on the use of convertible isocyanides[25] to widen the application potential of the generated dipeptide mimetics by allowing their use in peptide synthesis.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1. J. B. Bariwal, K. D. Upadhyay, A. T. Manvar, J. C. Trivedi, J. S. Singh, K. S. Jain and A. K. Shah, *Eur. J. Med. Chem.*, 2008, **43**, 2279-2290.

2. R. Kaur, R. Singh and K. Singh, *Chem. Biol. Lett.*, 2016, **3**, 18-31.

3. M. M. Mc Gee, S. Gemma, S. Butini, A. Ramunno, D. M. Zisterer, C. Fattorusso, B. Catalanotti, G. Kukreja, I. Fiorini, C. Pisano, C. Cucco, E. Novellino, V. Nacci, D. C. Williams and G. Campiani, *J. Med. Chem.*, 2005, **48**, 4367-4377.

4. H. Inoue, M. Konda, T. Hashiyama, H. Otsuka, K. Takahashi, M. Gaino, T. Date, K. Aoe and M. Takeda, *J. Med. Chem.*, 1991, **34**, 675-687.

5. K. S. Atwal, S. Z. Ahmed, D. M. Floyd, S. Moreland and A. Hedberg, *Bioorg. Med. Chem. Lett.*, 1993, **3**, 2797-2800.

6. J. Slade, J. L. Stanton, D. Ben-David and G. C. Mazzenga, *J. Med. Chem.*, 1985, **28**, 1517-1521.

7. J. W. Skiles, R. Sorcek, S. Jacober, C. Miao, P. W. Mui, D. McNeil and A. S. Rosenthal, *Bioorg. Med. Chem. Lett.*, 1993, **3**, 773-778.

8. M. Amblard, I. Daffix, G. Berge, M. Calmes, P. Dodey, D. Pruneau, J. L. Paquet, J. M. Luccarini, P. Belichard and J. Martinez, *J. Med. Chem.*, 1999, **42**, 4193-4201.

9. M. Amblard, I. Daffix, P. Bedos, G. Berge, D. Pruneau, J. L. Paquet, J. M. Luccarini, P. Belichard, P. Dodey and J. Martinez, *J. Med. Chem.*, 1999, **42**, 4185-4192.

10. M. Amblard, N. Raynal, M.-C. Averlant-Petit, C. Didierjean, M. Calmès, O. Fabre, A. Aubry, M. Marraud and J. Martinez, *Tetrahedron Lett.*, 2005, **46**, 3733-3735.

11. M. Amblard, P. Bedos, C. Olivier, I. Daffix, J.-M. Luccarini, P. Dodey, D. Pruneau, J.-L. Paquet and J. Martinez, *J. Med. Chem.*, 2000, **43**, 2382-2386.

12. P. Bedos, M. Amblard, G. Subra, P. Dodey, J.-M. Luccarini, J.-L. Paquet, D. Pruneau, A. Aumelas and J. Martinez, *J. Med. Chem.*, 2000, **43**, 2387-2394.

13. M. Amblard, M. Calmès, V. Roques, S. Tabet, A. Loffet and J. Martinez, *Org. Prep. Proced. Int.*, 2002, **34**, 405-415.

14. J. Gan and D. Ma, *Org. Lett.*, 2009, **11**, 2788-2790.

15. A. Dömling, *Chem. Rev.*, 2006, **106**, 17-89.

16. G. Koopmanschap, E. Ruijter and R. V. A. Orru, *Beilstein J. Org. Chem.*, 2014, **10**, 544-598.

17. T. M. A. Barlow, M. Jida, D. Tourwe and S. Ballet, *Org. Biomol. Chem.*, 2014, **12**, 6986-6989.

18. T. M. A. Barlow, M. Jida, K. Guillemyn, D. Tourwe, V. Caveliers and S. Ballet, *Org. Biomol. Chem.*, 2016, **14**, 4669-4677.

19. S. Caputo, A. Basso, L. Moni, R. Riva, V. Rocca and L. Banfi, *Beilstein J. Org. Chem.*, 2016, **12**, 139-143.

20. F. Y. Kwong and S. L. Buchwald, *Org. Lett.*, 2002, **4**, 3517-3520.

21. C. G. Bates, R. K. Gujadhur and D. Venkataraman, *Org. Lett.*, 2002, **4**, 2803-2806.

22. X. Moreau and J.-M. Campagne, *J. Organomet. Chem.*, 2003, **687**, 322-326.

23. T. Cierpicki, I. Zhukov, R. A. Byrd and J. Otlewski, *J. Magn. Reson.*, 2002, **157**, 178-180.

24. K. Van Rompaey, S. Ballet, C. Tömböly, R. De Wachter, K. Vanommeslaeghe, M. Biesemans, R. Willem and D. Tourwé, *Eur. J. Org. Chem.*, 2006, **2006**, 2899-2911.

25. G. van der Heijden, J. A. W. Jong, E. Ruijter and R. V. A. Orru, *Org. Lett.*, 2016, **18**, 984-987.