Journal Name

COMMUNICATION

# Synthesis and biological evaluation of a kabiramide C fragment modified with a WH2 consensus actin-binding motif as potential disruptor of the actin cytoskeleton†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Daniel J. Tetlow,[a] Steve J. Winder*[b] and Christophe Aïssa*[a]

**The F-actin depolymerisation potency of a fragment of kabiramide C was increased when modified with a WH2 consensus actin-binding motif LKKV. Despite its low affinity for actin monomers, a shorter analogous fragment not bearing LKKV was identified as a potent inhibitor of actin polymerisation and promoter of its depolymerisation, resulting in a loss of actin stress fibres in cells.**

The control of the actin cytoskeleton by actin-binding proteins (ABPs) is vital to a large number of fundamental cellular processes.[1] The Wiskott-Aldrich syndrome protein-homology domain 2 (WH2) is an actin-binding motif that binds actin between its subdomains 1 and 3.[2] WH2 is widely spread among ABPs and a representative example of actin/WH2 complex is depicted in Fig. 1a with the WH2 of the missing in metastasis protein (MIM, cyan and red cartoon).[3] In WH2, a key actin-recognition element consists of two basic residues (usually lysine), flanked by two hydrophobic residues.[2,4] Thus, a typical WH2 consensus actin-binding motif is LKKV or LKKT (Fig. 1a, red section of the cartoon).[2–4] Moreover, the actin cytoskeleton can be disrupted by several natural products,[5,6] among which kabiramide C (**1**) (Fig. 1b, green tubes),[7] aplyronine A and reidispongiolide A are prime examples. These marine toxins are structurally related and consist of a well-conserved aliphatic side chain attached to a macrolactone of varying size and structure. The crystal structures of the 1:1 complexes of these compounds and the actin monomer (G-actin) show considerable overlap of their binding site on actin with those of WH2 in actin/WH2 complexes, as illustrated with **1** in Fig. 1a.[7,8] Importantly, previous studies suggest that these compounds disrupt the actin cytoskeleton by slowly severing actin filaments (F-actin) and capping the shortened filament thus formed at their so-called barbed end.[8] In addition, the sub-nanomolar affinity of these natural toxins for G-actin suggests that the elongation of the filaments is also prevented by the incorporation of a G-actin/toxin complex at the barbed end of the filaments.[8] Remarkably, when truncated from the macrolactone ring, the lateral chain of aplyronine A and analogues thereof can still promote the depolymerisation of F-actin but with at least a ten-fold reduced potency as compared to the natural product.[9] Similarly, analogues of the lateral chain of reidispongiolide A, whereby the macrolactone ring is replaced with small apolar groups to enhance the hydrophobic interactions with actin, still display a sub-micromolar affinity for G-actin and are reported to sever F-actin.[10]

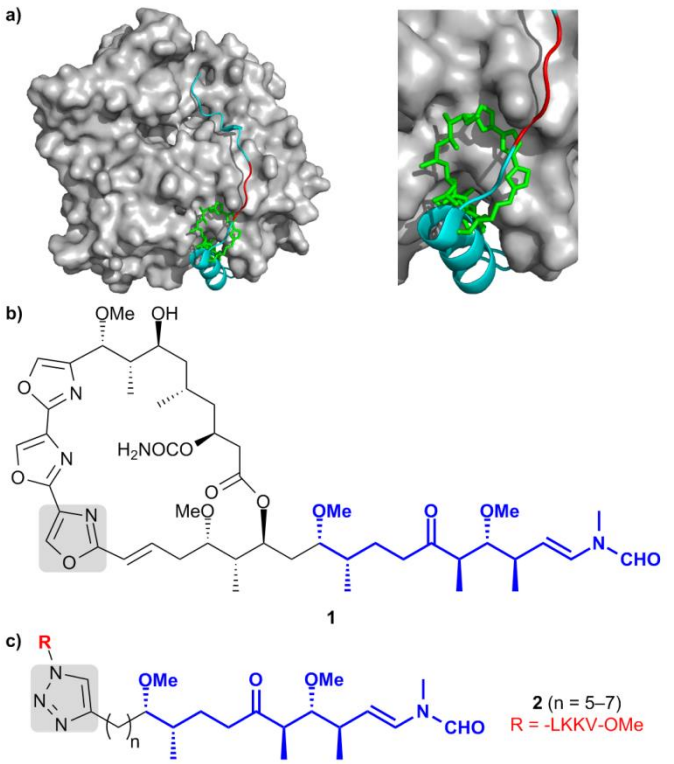
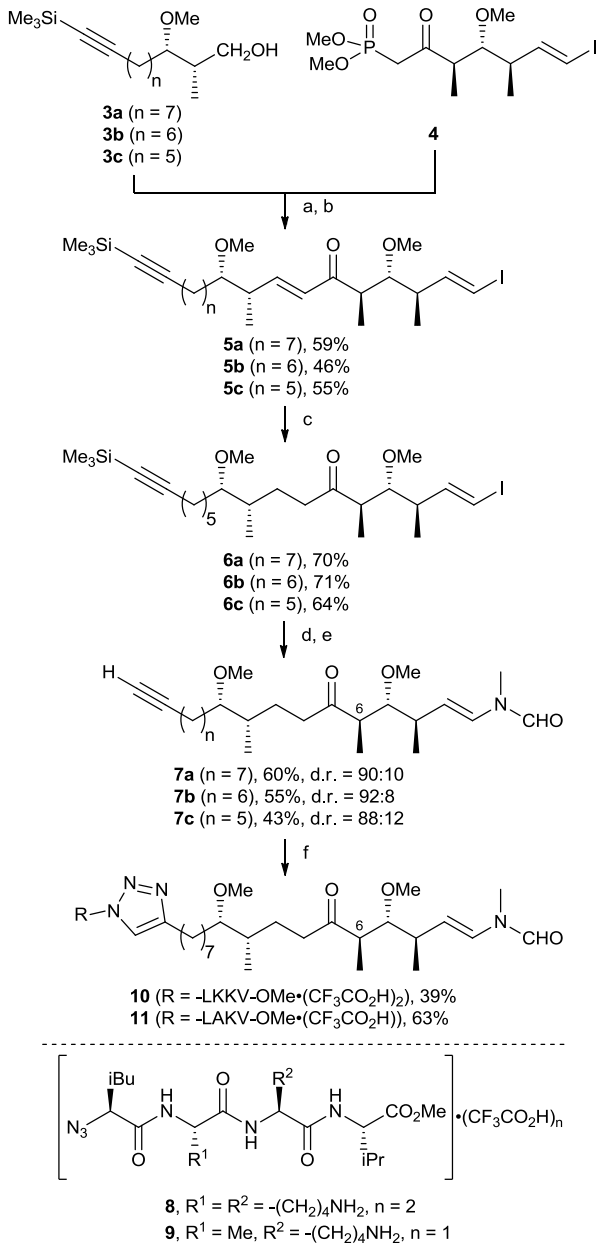



**Fig. 1** (a) Superimposition of actin/WH2 of MIM (1:1) complex (PDB: 2D1K, cyan and red cartoon) and actin/kabiramide C (1:1) complex (PDB: 1QZ5, green tubes). (b) Structure of kabiramide C (**1**). (c) Structure of hybrids **2** designed from the truncated side chain of **1** and the key actin-binding tetrapeptide LKKV.

[a] *Department of Chemistry, University of Liverpool, L69 7ZD, Liverpool, UK. E-Mail: aissa@liverpool.ac.uk*
[b] *Department of Biomedical Science, University of Sheffield, S10 2TN, UK. E-Mail: s.winder@sheffield.ac.uk*

†Electronic Supplementary Information (ESI) available: Synthetic procedures characterisation of compounds, bioassays results. See DOI: 10.1039/x0xx00000x




**Scheme 1** Synthesis of compounds. (a) **3a–c**, Dess-Martin periodinane (1.85 equiv), $NaHCO_3$ (2 equiv), $CH_2Cl_2$, rt, 0.25h; (b) i) **4** (1 equiv), $Ba(OH)_2$ (1.1 equiv), THF, 0.5h then ii) aldehyde obtained from **3a–c** (1 equiv), THF/water (40:1), rt, 1h; (c) $(CuHPPh_3)_6$ (0.33 equiv), water (10 equiv), toluene, rt, 1h; (d) CuI (0.2 equiv), 1,2-*trans*-cyclohexyldiamine (0.4 equiv), $K_3PO_4$ (2 equiv), MeNCHO (10 equiv), 1,4-dioxane, 80 °C, 16h; (d) $AgNO_3$ (4 equiv), THF/EtOH/water/2,6-lutidine (1:1:1:0.1), rt, 48h; (e) $CuSO_4$ (0.1 equiv), sodium L-ascorbate (0.2 equiv), **8** or **9**, *t*-BuOH, 35 °C, 48h.

In contrast to the strategy previously followed with the modified fragments of reidispongiolide A[10] and in view of the proximity of the WH2 consensus actin-binding motifs and **1** when in complex with G-actin (Fig. 1a),[3,7] we hypothesised that replacing the macrolactone moiety of **1** with the key actin-binding tetrapeptide LKKV in compounds **2** (Fig. 1c) would deliver hybrids that could trigger similar effects on the actin cytoskeleton as compared to **1**. According to this design, both actin-binding sections of **2** would be tethered by a chain of adjustable length (n = 5–7) and a triazole moiety that would replace one of the oxazole rings of **1** (highlighted in grey). We anticipated that this design could serve as a new basis for the future development of molecular tools to study the actin cytoskeleton, or drugs to fight diseases (e.g. cancer, viral infection, intraocular pressure and outflow) by targeting actin.[11]

Our approach towards hybrids **2** is illustrated in Scheme 1. Horner-Wadsworth-Emmons olefination between advanced fragments **3** and **4**[12,13] gave **5**. The reduction of the carbon-carbon double bond thus formed using Stryker's reagent[14] afforded intermediate **6**, with partial desilylation of the terminal alkyne (4%). The crude material was used without further purification in the next step. The *N*-methyl formamide moiety was installed using a copper-catalysed amidation reaction,[9b,15] and those basic conditions caused partial isomerisation at C(6).[16,17] The diastereomers were not separated and the synthesis was pursued nonetheless, as we assumed that the ratio of diastereomers would not prevent a meaningful evaluation of our hypothesis about the biological activity of the final compounds. Thus, cleavage of the trimethylsilyl group using $AgNO_3$[18] gave terminal alkyne **7a**. Attempts of the same cleavage using other conditions such as $K_2CO_3$ in MeOH or tetrabutylammonium fluoride in THF were unsuccessful and led to either incomplete reaction or decomposition, respectively. Analogues **7b** and **7c** were prepared in the same fashion, whereby uncontrolled desilylation of the triple bond (**6b** and **6c**, 12%) during the Stryker reduction and epimerisation at C(6) during the amidation reaction were also observed. Although the copper-catalysed formation of the triazole **2** using alkyne **7a** and azide **8** initially enabled us to obtain **10** has free bases,[19] we noticed a relatively rapid decomposition upon storage. Although the products of this decomposition were not identified, we suspected that it was linked to the presence of the free primary amino groups on the side chains of the two lysine residues. Accordingly, we modified the work-up procedure of the copper-catalysed 1,3-dipolar addition between **7a** and **8** in order to isolate **10** as a bis-TFA salt. In order to assess whether a putative enhancement of actin-binding properties could be attributed to the specific tetrapeptide LKKV, the analogous control substrate **11** was prepared from **7a** and **9**, whereby the LKKV fragment was replaced with LAKV. Finally, although we initially intended to prepare analogues of **10** from **7b** and **7c**, the results from the initial comparison of the affinity for G-actin of **7a** and **10** did not encourage us to pursue this endeavour.

Thus, a rapid comparison of the G-actin binding properties of **7a**, **10** and **11** was conducted by scanning the fluorescence intensity of a 1 µM solution of Prodan-G-actin in the absence or presence of 25 µM solutions of the compounds (Fig. 2).[10,20] Contrary to our initial hypothesis that modifying **7a** with the LKKV fragment would increase the affinity of **10** for G-actin, the scan did not reveal any evidence of such effect. In contrast, a minor increase of affinity was observed for control compound **11** which bears the LAKV fragment. Moreover, shortening the aliphatic chain in **7a**–**7c** led to compounds with increasingly lower affinity for G-actin.

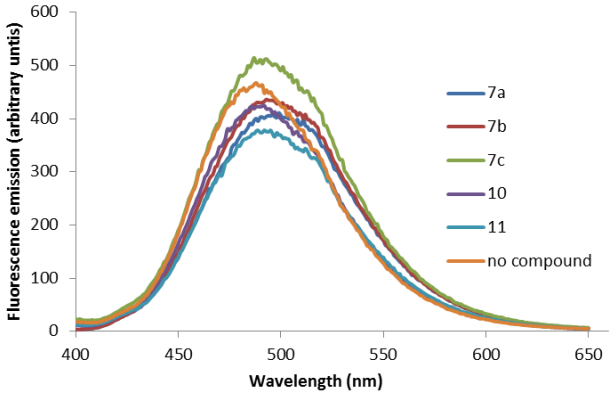



**Fig. 2** Fluorescence emission spectra of 1 µM Prodan-G-actin in the presence of 25 µM compounds **7a–7c**, **10**, and **11**.

**Table 1** Potency of compounds **7a–c**, **10** and **11** in regard to the inhibition of G-actin polymerisation (A), promotion of F-actin depolymerisation (B) and to the disruption of actin cytoskeleton in rat fibroblast cells after 2h (C) and 24h (D)[a]

| Compound | A[b] | B[b] | C | D |
|---|---|---|---|---|
| **7a** | 10 (-) | 5 (+) | ±[d] | 20 |
| **7b** | 1 (+) | 5 (+) | 20 | 20 |
| **7c** | 1 (-) | 5 (+) | ±[d] | 0.8 |
| **10** | 10 (+) | 1.5 (+) | 20 | ±[d] |
| **11** | ±[c] | 5 (+) | 20 | ±[d] |

[a] Minimal concentration (µM) of compound for which a significant effect was observed. [b] Inhibiting and promoting effects are indicated by a minus and plus sign, respectively. [c] No effect at 10 µM. [d] No effect at 20 µM.

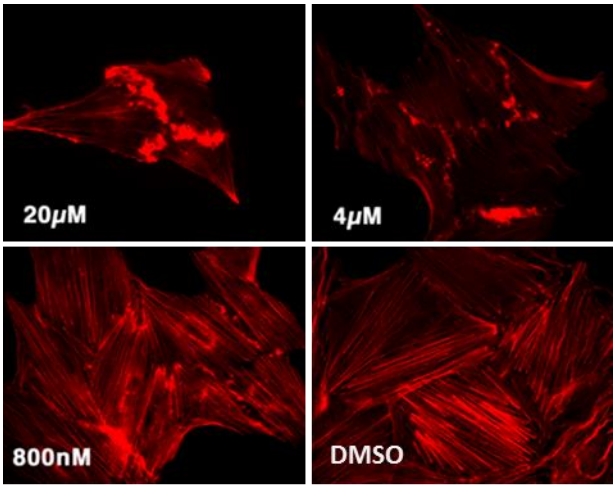



**Fig. 3** Effect of **7c** on the actin cytoskeleton in REF52 rat embryo fibroblast cells 24h after they have been treated with 0.8–20 µM solutions of the compound.

The potency of all compounds was nevertheless evaluated in G-actin polymerisation[21] and F-actin depolymerisation assays[22] as well as whole cell assays.[23] In the polymerisation assay of 2 µM pyrenyl G-actin, the initial rates of polymerisation were calculated for compounds in the initial linear phase of polymerisation up to 20 minutes. The minimum concentration that reduced the polymerisation rate by 10% compared to DMSO control was deemed significant. The quantification of the depolymerisation assay was performed by measuring the amount of actin in the supernatant obtained after incubation of 6 µM F-actin with **7a–c**, **10**, and **11**. A compound was deemed to have a significant effect at a concentration that resulted in 5% of actin in the supernatant fraction whereas between 1 and 2% of actin was in the supernatant fraction when F-actin was incubated with DMSO only. The results of those assays are summarised in Table 1. They demonstrated that despite its low affinity for G-actin **7c** is a strong inhibitor of G-actin polymerisation whilst it also promotes the depolymerisation of F-actin. Treating REF52 rat embryo fibroblast cells with compound **7c** led to a concentration-dependent loss of actin stress fibres and actin stress fibre organisation (Fig. 3). Compared to DMSO alone, 800 nM **7c** caused an increased accumulation of disorganised F-actin clouds in the perinuclear region, with a progressive loss of stress fibres and stress fibre organisation with increased concentrations up to 20 µM. Furthermore, the numbers of cells remaining attached to the culture dish was also reduced as the concentration of **7c** increased, probably as a consequence of the loss of stress fibre-mediated cell adhesion contacts to the substratum. At 20 µM **7c**, in any remaining adherent cells, F-actin was mostly concentrated in dense accumulations at the cell periphery adjacent to other cells. Among compounds **7a–7c**, **7c** is the most potent inhibitor of G-actin polymerisation and the most potent promoter of F-actin depolymerisation (Table 1). Moreover, attaching the LKKV fragment to the polypropionate chain of **7a** increased the potency of **10** to act as promoter of F-actin depolymerisation. This effect appears to be attributable to the LKKV fragment as replacing even only one of the two lysine residues with a non-basic residue (**11**, Table 1) led to a complete loss of potency in all assays. However, both **10** and **11** failed to elicit any significant effects in whole-cell assays, which might be due to issues of cell penetration.

In conclusion, and contrary to our initial hypothesis, modifying a simplified side chain of kabiramide C with a WH2 actin-binding motif does not enhance the affinity of the compound thus obtained for G-actin. However, the F-actin depolymerisation potency is increased by this modification and this effect appears to be specific to the LKKV fragment. Moreover, the remarkable effects observed for **7c** in the assays described above cannot be explained by the sequestration of actin monomers due to its low of affinity for G-actin, in contrast to the modified side chains of reidispongiolide A that exhibit a remarkable sub-micromolar affinity for G-actin.[10b] Further investigations are required to delineate more precisely the mechanism of action of this compound.

We are grateful to the Leverhulme Trust (research grant RPG-198) for financial support and to Dr Neil Berry for his help during the preparation of Fig. 1a.

## Notes and references

1 (a) C. G. dos Remedios, D. Chhabra, M. Kekic, I. V. Dedova, M. Tsubakihara, D. A. Berry and N. J. Nosworthy, *Physiol. Rev.* 2003, **83**, 433. (b) V. O. Paavilainen, E. Bertling, S. Falck and P. Lappalainen, *Trends. Cell. Biol.*, 2004, **14**, 386. (c) R.

Dominguez, *Trends Biochem. Sci.*, 2004, **29**, 572. (d) S. J. Winder and K. R. Ayscough, *J. Cell Sci.*, 2005, **118**, 651.
2 D. Chereau, F. Kerff, P. Graceffa, Z. Grabarek, K. Langsetmo and R. Dominguez, *Proc. Natl. Acad. Sci. U.S.A.*, 2005, **102**, 16644.
3 S. H. Lee, F. Kerff, D. Chereau, F. Ferron, A. Klug and R. Dominguez, *Structure*, 2007, **15**, 145.
4 (a) E. Paunola, P. K. Mattila, P. Lappalainen, *FEBS Lett.*, 2002, **513**, 92. (b) B. Qualmann, M. K. Kessels, *Trends Cell. Biol.*, 2009, **19**, 276.
5 (a) K.-S. Yeung and I. Paterson, *Angew. Chem. Int. Ed.*, 2002, **41**, 4632. (b) J. S. Allingham, V. A. Klenchin and I. Rayment, *Cell. Mol. Life. Sci.*, 2006, **63**, 2119.
6 For selected examples not covered in references 5, see: (a) A. V. Statsuk, R. Bai, J. L. Baryza, V. A. Verma, E. Hamel, P. A. Wender and S. A. Kozmin, *Nature Chem. Biol.*, 2005, **1**, 383. (b) A. Fürstner, C. Nevado, M. Waser, M. Tremblay, C. Chevrier, F. Teplý, C. Aïssa, E. Moulin and O. Müller, *J. Am. Chem. Soc.*, 2007, **129**, 9150. (c) R. Tannert, L.-G. Milroy, B. Ellinger, T.-S. Hu, H.-D. Arndt and H. Walmann, *J. Am. Chem. Soc.*, 2010, **132**, 3063. (d) D. Herkommer, S. Dreisigacker, G. Sergeev, F. Sasse, H. Gohlke and Dirk Menche, *ChemMedChem*, 2015, **10**, 470.
7 V. A. Klenchin, J. S. Allingham, R. King, J. Tanaka, G. Marriott and I. Rayment, *Nature Struct. Biol.*, 2003, **10**, 1058.
8 (a) J. Tanaka, Y. Yan, J. Choi, J. Bai, V. A. Klenchin, I. Rayment and G. Marriott, *Proc. Natl. Acad. Sci. U.S.A.*, 2003, **100**, 13851; (b) J. S. Allingham, A. Zampella A, M. V. D'Auria and I. Rayment, *Proc. Natl. Acad. Sci. U.S.A.*, 2005, **102**, 14527; (c) K. Hirata, S. Muraoka, K. Suenaga, T. Kuroda, K. Kato, H. Tanaka, M. Yamamoto, M. Takata, K. Yamada and H. Kigoshi, *J. Mol. Biol.*, 2006, **356**, 945.
9 (a) H. Kigoshi, K. Suenaga, M. Takagi, A. Akao, K. Kanematsu, N. Kamei, Y. Okugawa and K. Yamada, *Tetrahedron*, 2002, **58**, 1075; (b) K. Kitamura, T. Teruya, T. Kuroda, H. Kigoshi and K Suenaga, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 1896.
10 (a) R. D. Perrins, G. Cecere, I. Paterson and G. Marriott, *Chem. Biol.*, 2008, **15**, 287; (b) J. H. Pereira, C. Petchprayoon, A. C. Hoepker, N. W. Moriarty, S. J. Fink, G. Cecere, I. Paterson, P. D. Adams and G. Marriott, *ChemMedChem*, 2014, **9**, 2286.
11 (a) I. Spector, F. Braet, N. V. Shochet and M. R. Bubb, *Microsc. Res. Techniq.*, 1999, **47**, 18. (b) F. Braet, L. Soon, K. Vekemans, P. Thordarson and I. Spector *Protein Reviews*, ed. C. G. dos Remedios and D. Chhabra, Springer, New York, 2008, **8**, 37.
12 See the supporting information for details of the preparation of intermediates **3** and **4**.
13 (a) I. Paterson, K.-S. Yeung and J. B. Smaill, *Synlett*, 1993, 774. (b) S. K. Chattopadhyay, G. Pattenden, *Tetrahedron Lett.*, 1995, **36**, 5271; (c) I. Paterson, K.-S. Yeung, C. Watson, R. A. Ward and P. A. Wallace, *Tetrahedron*, 1998, **54**, 11935; (d) I. Paterson, S. B. Blakey and C. J. Cowden, *Tetrahedron Lett.*, 2002, **43**, 6005; (e) A. Zampella, V. Sepe, R. D'Orsi, R. Bifulco, C. Bassarello, M. V. D'Auria, *Tetrahedron: Asymmetry*, 2003, **14**, 1787; (f) T. J. Hoffman, A. Kolleth, J. H. Rigby, S. Arseniyadis and J. Cossy, *Org. Lett.*, 2010, **12**, 3348.
14 W. S. Mahoney, D. M. Brestensky and J. M. Stryker, *J. Am. Chem. Soc.*, 1988, **110**, 291.
15 A. Klaspars, J. C. Antilla, X. Huang and S. L. Buchwald, *J. Am. Chem. Soc.*, 2001, **123**, 7727.
16 M. Ying and W. R. Roush, *Tetrahedron*, 2011, **67**, 10274.
17 A doublet of quartets is observed for H(6) in $^1$H NMR (**7a**: $J_{6\text{-}5}$ = 9.7 Hz and $J_{6\text{-Me}}$ = 7.1 Hz, **7b**: $J_{6\text{-}5}$ = 9.6 Hz and $J_{6\text{-Me}}$ = 7.0 Hz, **7c**: $J_{6\text{-}5}$ = 9.5 Hz and $J_{6\text{-Me}}$ = 7.0 Hz). Accordingly, the configuration at C(6) for the major diastereomer of **7a**–**7c**, **10** and **11** is tentatively assumed to be as depicted in Scheme 1 by comparison with similar compounds. (a) I. Paterson and K.-S. Yeung, *Tetrahedron Lett.*, 1993, **34**, 5347. (b) E. Fleury, M.-I. Lannou, O. Bistri. F. Sautel, G. Massiot, A. Pancrazi and J. Ardisson, *J. Org. Chem.*, 2009, **74**, 7034.
18 E. M. Carreira and J. Du Bois, *J. Am. Chem. Soc.*, 1995, **117**, 8106.
19 A. Paul A, H. Bittermann and P. Gmeiner, *Tetrahedron*, 2006, **62**, 8919.
20 G. Marriott, K. Zechel and T. M. Jovin, *Biochemistry*, 1988, **27**, 6214.
21 M. Pfuhl, S. J. Winder and A. Pastore, *EMBO J.*, 1994, **13**, 1782.
22 S. J. Winder, L. Hemmings, S. K. Maciver, S. J. Bolton, J. M. Tinsley, K. E. Davies, D. R. Critchley and J. Kenderick-Jones, *J Cell Sci.*, 1995, **108**, 63.
23 Y.-J. Chen, H. J. Spence, J. M. Cameron, T. Jess, J. L. Ilsley and S. J. Winder, *Biochem J.*, 2003, **375**, 329.