Bioorganic & Medicinal Chemistry Letters 26 (2016) 262–264

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Total synthesis of a biotinylated rocaglate: Selective targeting of the translation factors eIF4AI/II

Jennifer M. Chambers [a], Lisa M. Lindqvist [b,c,*], G. Paul Savage [d], Mark A. Rizzacasa [a,*]

[a] School of Chemistry, The Bio21 Institute, The University of Melbourne, Melbourne, Victoria 3010, Australia
[b] Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia
[c] Department of Medical Biology, The University of Melbourne, Parkville, Victoria 3010, Australia
[d] CSIRO Molecular and Health Technologies, Bayview Avenue, Victoria 3168, Australia

ARTICLE INFO

Article history:
Received 17 September 2015
Revised 26 October 2015
Accepted 12 December 2015
Available online 14 December 2015

Keywords:
Methyl rocaglate
Silvestrol
Biotin tag
eIF4A
Pulldown

ABSTRACT

The total synthesis of a biotinylated derivative of methyl rocaglate is described. This compound was accessed from synthetic methyl rocaglate (**2**) via formation of the propargyl amide and subsequent click reaction with a biotin azide. Affinity purification revealed that biotinylated rocaglate (**8**) and methyl rocaglate (**2**) bind with high specificity to translation factors eIF4AI/II. This remarkable selectivity is in line with that found for the more complex rocaglate silvestrol (**3**).

Of the more than 100 flavaglines known, about 60 of them are cyclopenta[*b*]benzofuran type compounds, with many of these natural products exhibiting insecticidal and antiproliferative properties.[1,2] The first cyclopenta[*b*]benzofuran derivative, rocaglamide (**1**), was isolated from *Aglaia elliptifolia* in 1982 and the structure was solved by single crystal X-ray structural analysis.[3] A large number of other rocaglates have been isolated including aglafoline (**2**) from *Aglaia elliptifolia* in 1992[4] (see Fig. 1).

Compound **2** was also isolated from *Aglaia odorata* in 1993 and given the name (−)-methyl rocaglate.[5] The more complex and highly active 1,4-dioxane containing rocaglates silvestrol (**3**) and the C5‴ epimer episilvestrol (**4**), were isolated by two independent groups from both *Aglaia foveolata* and *Aglaia leptantha*.[6–8] Silvestrol (**3**) shows potent anticancer activity,[9] in particular against CLL,[10] and can induce multiple forms of cell death, which varies between cell types.[11]

Two conflicting reports have been published concerning the protein targets of rocaglate natural products such as rocaglamide (**1**). Li-Weber and co-workers found that a rocaglaol derivative demonstrated similar IC_{50} and effects on growth inhibition of Jurkat cells as rocaglamide (**1**).[12] To find the cellular targets, rocaglamide (**1**) was conjugated to Affi-Gel® beads and performed a pull-down assay. Two proteins were found to bind to rocaglamide (**1**) and were identified as prohibitin (PHB) 1 and 2 by GC–MS and Western blot analysis. It was therefore concluded that rocaglamide binding to PHB prevented it from interacting with cRaf, and thus inhibited translation indirectly by blocking the Raf–MEK–ERK pathway. However, the translation initiation factor eIF4A was also identified as the primary protein target of rocaglamide (**1**) using a rocaglamide analog.[13] Indeed, previous work suggested that PHB 1/2 is not likely to be involved in the mode of action of translation inhibition, as rocaglamide compounds have retained translational activity in cellular extracts, which do not have a functional Raf–MEK–ERK pathway, which is responsible for communication between extracellular receptor and the nucleus.[9] We have recently described the total synthesis of a biotinylated derivative of episilvestrol (**4**) and demonstrated that this binds only to eIF4AI/II.[14] We now describe the total synthesis of biotinylated rocaglate and demonstrate that methyl rocaglate (**2**) and rocaglamide (**1**) have a similar selectivity for eIF4AI/II as episilvestrol.

The synthesis of biotinylated rocaglate began with methyl rocaglate (**2**), which was accessed via total synthesis.[8b] using a [3+2]-cycloaddition of an oxidopyrylium as pioneered by Porco.[15,16] Hydrolysis of **2** with aqueous KOH gave rocagloic acid (**5**)[16e] which was converted into the propargyl amide (**6**) by coupling with propargyl amine. A Cu mediated click reaction with the

* Corresponding authors. Tel.: +61 3 9345 2974; fax: +61 3 9347 0852 (L.M.L.); tel.: +61 3 8344 2397; fax: +61 3 9347 8396 (M.A.R.).
E-mail addresses: lindqvist@wehi.edu.au (L.M. Lindqvist), masr@unimelb.edu.au (M.A. Rizzacasa).

http://dx.doi.org/10.1016/j.bmcl.2015.12.045
0960-894X/

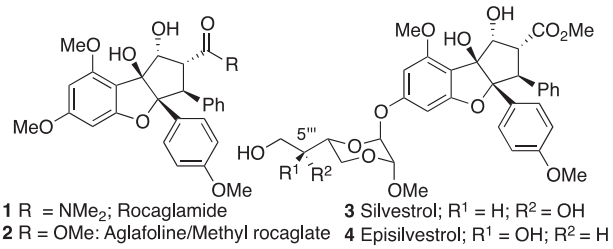
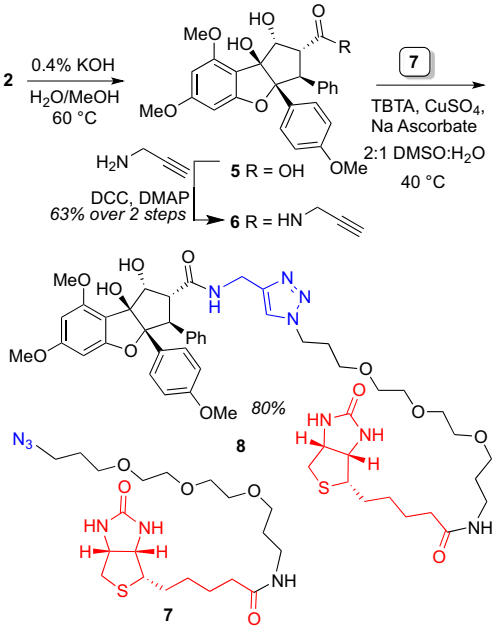


Figure 1. Structures of rocaglamide (**1**), aglafoline/methyl rocaglate (**2**), silvestrol (**3**) and episilvestrol (**4**).

biotin azide[13,17] in the presence of TBTA[18] afforded biotinylated rocaglate **8** in good yield (see Scheme 1).

All rocaglamide derivatives, along with the parent methyl rocaglate (**2**), were tested using an in vitro translation assay[9,14,19] (Fig. 2). While rocagloic acid (**5**) showed a similar activity as the methyl rocaglate (**2**), biotinylated rocaglamide **8** was ~2 times less active. The propargyl amide **6** demonstrated a surprisingly high level of translation inhibition. Compared to episilvestrol (**4**) at an identical concentration, propargyl amide (**6**) was only ~4 times less active. This increased potency agrees with literature reports that C2 amide[20] and hydroxamate[21] analogs of methyl rocaglate (**2**) often have activity on the order of episilvestrol (**4**).

To determine if rocaglamide was as specific as silvestrol, or if the sugar moiety added specificity to the compound, a pulldown assay was performed by incubating RNAse A-treated mouse embryonic fibroblast (MEF) lysates (to remove in-direct RNA binding proteins) as previously described with beads bound with either biotinylated episilvestrol, biotinylated rocaglate (**8**), or a negative control biotin only.[14] When proteins were eluted with free episilvestrol after extensive washing from beads bound with biotinylated episilvestrol, only one intense band eluted specifically from biotinylated episilvestrol and was absent in the biotin-only negative control (Fig. 3A, compare lane 9–10). This band was previously identified by mass spectrometry as eIF4AI/II.[14] One other band of a



Scheme 1. Synthesis of biotinylated rocaglate **8**.

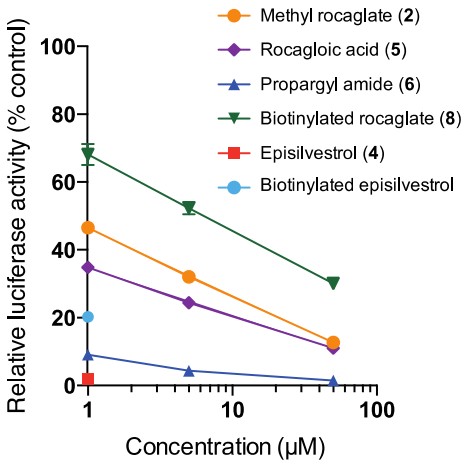


Figure 2. Cap-dependent in vitro translation assay of methyl rocaglate and derivatives. Reactions were performed in rabbit reticulocyte lysate programmed with capped Firefly luciferase/HCV/Renilla luciferase mRNA. Data are represented as Firefly luciferase/Renilla luciferase (FF/Ren) relative to vehicle (DMSO) controls ($n = 3$). HCV-driven Renilla served as an internal control.

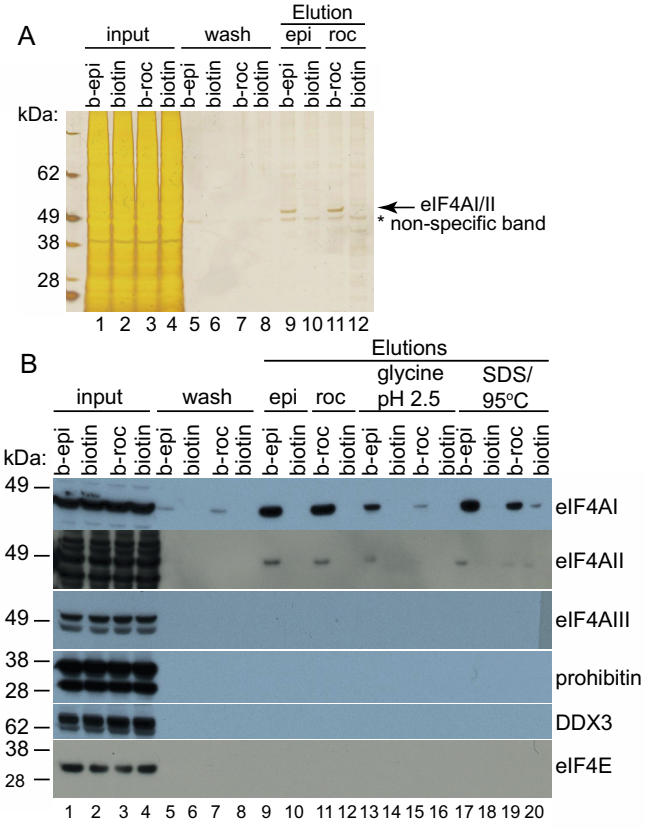


Figure 3. eIF4AI/II are the major targets of biotinylated episilvestrol and biotinylated rocaglate (8). (A) Streptavidin sepharose pulldowns with **8** (b-roc), biotinylated episilvestrol (b-epi), or free biotin control were washed (lanes 5–8) and eluted with either 50 μM episilvestrol (epi) (lanes 9–10) or methyl rocaglate (roc) (**2**) (lanes 11–12). Samples were separated by NuPAGE and visualized by silver stain. (B) Streptavidin sepharose pulldowns with **8** (b-roc), biotinylated episilvestrol (b-epi), or free biotin control were washed (lanes 5–8) and eluted sequentially with either 50 μM episilvestrol (epi)(lanes 9–10) or methyl rocaglate (roc) (**2**) (lanes 11–12), then with low pH glycine (lanes 13–16), and finally boiled in SDS (lanes 17–20). Western blot probed with antibodies raised against the indicated proteins.

slightly reduced size was also visible, but this was non-specific to biotin-episilvestrol, as it was equally abundant in the biotin-only negative controls (Fig. 3A, lanes 10 and 12).

Strikingly, a very similar banding pattern occurred when a parallel pulldown was performed with biotinylated rocaglate (**8**) and eluted with free parent compound methyl rocaglate (**2**) (Fig. 3A, compare lanes 11 and 12), indicating that methyl rocaglate (**2**) and rocaglamide (**1**) have the same specificity as episilvestrol (**4**). This result was confirmed by Western blot analysis (Fig. 3B), which revealed that only eIF4AI/II but not prohibitin (PHB 1/2), eIF4AIII, DDX3, and eIF4E bound to biotinylated rocaglate (**8**), similar to parallel pulldowns done with biotinylated episilvestrol.[14] This was determined by sequentially eluting MEF lysate-incubated beads with parental compound (lanes 9–12), followed by low pH (lanes 13–16), and then the more stringent condition of boiling in SDS (lanes 17–20). This indicates that eIF4AI/II is probably the major direct protein target of cyclopenta[*b*]benzofurans rocaglamide (**1**), methyl rocaglate (**2**) and the rocaglate family of natural products and that the dioxylanoxy group present in silvestrol (**3**) and episilvestrol (**4**) is not critical for this selective binding.

Acknowledgments

We thank the Australian Research Council-Discovery Grants Scheme for funding. LML holds an NHMRC Peter Doherty Early Career Fellowship (1035502). Research on this subject is supported by the Victorian State Government Operational Infrastructure Support and Australian Government NHMRC IRIISS to WEHI (LML).

Supplementary data

Supplementary data (full experimental details, characterization data and copies of NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.12.045.

References and notes

1. Pannell, C. M. In *Kew Bull., Addit. Ser.*; Royal Botanic Gardens, Kew: London, 1992; Vol. 16, p 379.
2. (a) Ebada, S. S.; Lajkiewicz, N.; Porco, J. A., Jr.; Li-Weber, M.; Proksch, P. *Prog. Chem. Org. Nat. Prod.* **2011**, *94*, 1; (b) Pan, L.; Woodard, J. L.; Lucas, D. M.; Fuchs, J. R.; Kinghorn, A. D. *Nat. Prod. Rep.* **2014**, *31*, 924; (c) Ebada, S. S.; Lajkiewicz, N.; Porco, J. A.; Li-Weber, M.; Proksch, P. *Prog. Chem. Org. Nat. Prod.* **2011**, *94*, 1.
3. King, M. L.; Chiang, C. C.; Ling, H. C.; Fugita, E.; Ochiai, M.; McPhail, A. T. *J. Chem. Soc., Chem. Commun.* **1982**, 1150.
4. Ko, F. N.; Wu, T. S.; Liou, M. J.; Huang, T. F.; Teng, C. M. *Eur. J. Pharmacol.* **1992**, *218*, 129.
5. Ishibashi, F.; Satasook, C.; Isman, M. B.; Towers, G. H. N. *Phytochemistry* **1993**, *32*, 307.
6. Meurer-Grimes, B. M.; Yu, J.; Vario, G. L. WO 2002002566, A1 20020110, 2004, 66pp.
7. Hwang, B. Y.; Su, B.-N.; Chai, H.; Mi, Q.; Kardono, L. B. S.; Afriastini, J. J.; Riswan, S.; Santarsiero, B. D.; Mesecar, A. D.; Wild, R.; Fairchild, C. R.; Vite, G. D.; Rose, W. C.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Swanson, S. M.; Kinghorn, A. D. *J. Org. Chem.* **2004**, *69*, 3350. Correction: *J. Org. Chem.* **2004**, *69*, 6156.
8. For the total synthesis of **3** and **4** see: (a) El Sous, M.; Khoo, M. L.; Holloway, G.; Owen, D.; Scammells, P. J.; Rizzacasa, M. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 7835; (b) Adams, T. E.; El Sous, M.; Hawkins, B. C.; Hirner, S.; Holloway, G.; Khoo, M. L.; Owen, D. J.; Savage, G. P.; Scammells, P. J.; Rizzacasa, M. A. *J. Am. Chem. Soc.* **2009**, *131*, 1607; Total synthesis of **3**: (c) Gerard, B.; Cencic, R.; Pelletier, J.; Porco, J. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 7831; Total synthesis of 2‴, 5‴-diepisilvestrol: (d) Chambers, J. M.; Huang, D. C. S.; Lindqvist, L. M.; Savage, G. P.; White, J. M.; Rizzacasa, M. A. *J. Nat. Prod.* **2012**, *75*, 1500.
9. Bordeleau, M. E.; Robert, F.; Gerard, B.; Lindqvist, L.; Chin, S. M. H.; Wendel, H. G.; Brem, B.; Greger, H.; Lowe, S. W.; Porco, J. A., Jr.; Pelletier, J. *J. Clin. Invest.* **2008**, *118*, 1.
10. Lucas, D. M.; Edwards, R. B.; Lozanski, G.; West, D. A.; Shin, J. D.; Vargo, M. A.; Davis, M. E.; Rozewski, D. M.; Johnson, A. J.; Su, B. N.; Goettl, V. M.; Heerema, N. A.; Lin, T. S.; Lehman, A.; Zhang, X. L.; Jarjoura, D.; Newman, D. J.; Byrd, J. C.; Kinghorn, A. D.; Grever, M. R. *Blood* **2009**, *113*, 4656.
11. Lindqvist, L. M.; Vikstrom, I.; Chambers, J. M.; McArthur, K.; Anderson, M. A.; Henley, K. J.; Happo, L.; Cluse, L.; Johnstone, R. W.; Roberts, A. W.; Kile, B. T.; Croker, B. A.; Burns, C. J.; Rizzacasa, M. A.; Strasser, A.; Huang, D. C. S. *Cell Death Dis.* **2012**, *3*, e409.
12. Polier, G.; Neumann, J.; Thuaud, F.; Ribeiro, N.; Gelhaus, C.; Schmidt, H.; Giaisi, M.; Kohler, R.; Muller, W. W.; Proksch, P.; Leippe, M.; Janssen, O.; Desaubry, L.; Krammer, P. H.; Li-Weber, M. *Chem. Biol.* **2012**, *19*, 1093.
13. Sadlish, H.; Galicia-Vazquez, G.; Paris, C. G.; Aust, T.; Bhullar, B.; Chang, L.; Helliwell, S. B.; Hoepfner, D.; Knapp, B.; Riedl, R.; Roggo, S.; Schuierer, S.; Studer, C.; Porco, J. A., Jr.; Pelletier, J.; Movva, N. R. *ACS Chem. Biol.* **2013**, *8*, 1519.
14. Chambers, J. M.; Lindqvist, L. M.; Webb, A.; Huang, D. C. S.; Savage, G. P.; Rizzacasa, M. A. *Org. Lett.* **2013**, *15*, 1406.
15. Total syntheses of **1** and **2** see: (a) Gerard, B.; Jones, G., II; Porco, J. A., Jr. *J. Am. Chem. Soc* **2004**, *126*, 13620; (b) Gerard, B.; Sangji, S.; O'Leary, D. J.; Porco, J. A., Jr. *J. Am. Chem. Soc.* **2006**, *128*, 7754.
16. For other total syntheses of **1** and **2** see: (a) C2,3-epimer: Kraus, G. A.; Sy, J. O. *J. Org. Chem.* **1989**, *54*, 77; (b) Trost, B. M.; Greenspan, P. D.; Yang, B. V.; Saulnier, M. G. *J. Am. Chem. Soc.* **1990**, *112*, 9022; (c) Davey, A. E.; Schaeffer, M. J.; Taylor, R. J. K. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2657; (d) Dobler, M. R.; Bruce, I.; Cederbaum, F.; Cooke, N. G.; Diorazio, L. J.; Halla, R. G.; Irving, E. *Tetrahedron Lett.* **2001**, *42*, 8281; (e) Malona, J. A.; Cariou, K.; Frontier, A. J. *J. Am. Chem. Soc.* **2009**, *131*, 7560; (f) Magnus, P.; Freund, W. A.; Moorhead, E. J.; Rainey, T. *J. Am. Chem. Soc.* **2012**, *134*, 6140; (g) Zhou, Z.; Tius, M. A. *Angew. Chem., Int. Ed.* **2015**, *54*, 6037.
17. Stewart, S. G.; Braun, C. J.; Polomska, M. E.; Karimi, M.; Abraham, L. J.; Stubbs, K. A. *Org. Biomol. Chem.* **2010**, *8*, 4059.
18. Donnelly, P. S.; Zanatta, S. D.; Zammit, S. C.; White, J. M.; Williams, S. J. *Chem. Commun.* **2008**, 2459.
19. Novac, O.; Guenier, A. S.; Pelletier, J. *Nucleic Acids Res.* **2004**, *32*, 902.
20. Liu, T.; Nair, S. J.; Lescarbeau, A.; Belani, J.; Peluso, S.; Conley, J.; Tillotson, B.; O'Hearn, P.; Smith, S.; Slocum, K.; West, K.; Helble, J.; Douglas, M.; Bahadoor, A.; Ali, J.; McGovern, K.; Fritz, C.; Palombella, V. J.; Wylie, A.; Castro, A. C.; Tremblay, M. R. *J. Med. Chem.* **2012**, 8859.
21. Rodrigo, C. M.; Cencic, R.; Roche, S. P.; Pelletier, J.; Porco, J. A., Jr. *J. Med. Chem.* **2012**, *55*, 558.