Chemical Science

ROYAL SOCIETY OF CHEMISTRY

EDGE ARTICLE

Check for updates

All publication charges for this article have been paid for by the Royal Society of Chemistry

Total synthesis of the antibacterial polyketide natural product thailandamide lactone†

Himangshu Sharma,[a] Joyanta Mondal,[a] Ananyo K. Ghosh,[b] Ritesh Ranjan Pal [ID] [b] and Rajib Kumar Goswami [ID] *[a]

Stereoselective total synthesis of the structurally intriguing polyketide natural product thailandamide lactone was accomplished, and done so using a convergent approach for the first time to the best of our knowledge. The key features of this synthesis included use of a Crimmins acetate aldol reaction, Evans methylation, Urpi acetal aldol reaction, Sharpless asymmetric epoxidation and subsequent γ-lactonization for the installation of six asymmetric centers and the use of the Negishi reaction, Julia-Kocienski olefination, cross metathesis, HWE olefination and intermolecular Heck coupling for construction of a variety of unsaturated linkages. Pd(ɪ)-based Heck coupling was introduced, for the first time to the best of our knowledge, quite efficiently to couple the major eastern and sensitive western segments of the molecule. The antibacterial activity of thailandamide lactone was also evaluated.

Introduction

During the mining of the genome of *Burkholderia thailandensis*, a bacterium isolated from rice paddies in central and northeastern Thailand, Hertweck and co-workers[1] in 2008 first observed the labile polyene polyketide thailandamide A (**1**, Fig. 1), albeit in minute quantities. To better understand thailandamide biosynthesis, the silent *tha* PKS-NRPS gene cluster of *B. thailandensis* was activated by a research group in 2010 through manipulation of a quorum sensing (QS) regulatory system that produced a mutant with a dramatically altered metabolic profile.[2] This process resulted in the isolation of the structurally challenging new polyketide thailandamide lactone (**2**, Fig. 1), which was not detected in wild type broth initially. Moreover, the production of thailandamide A (**1**) was significantly greater here than when the wild type was used.[2] Later, the same group developed an elegant biosynthetic route yielding the first total synthesis of thailandamide A; this development enabled them to find another unstable metabolite, namely thailandamide B (**3**, Fig. 1), a geometrical isomer of thailandamide A.[3] Broad biological screening of thailandamide A revealed its selective and potential inhibitory activity against various pathogenic Gram-positive and Gram-negative bacteria with a specific mode of action.[4] However, the antibacterial activity of thailandamide lactone and thailandamide B

remained undisclosed. The highly challenging architectural features and natural scarcity of thailandamide lactone and lack of a synthetic route to this lactone—together with our continual interest in natural products chemistry[5]—encouraged us to seek out its total synthesis. Structurally, thailandamide lactone[2] is a linear polyene polyketide where a tetraene conjugated with a γ-butyrolactone is fused with a conjugated triene through an enolized dione moiety. It consists of six asymmetric centers including a quaternary center at one terminus of the molecule and a phenolic moiety at the other terminus. Herein, we report a convergent and highly modular route for the first total synthesis of thailandamide lactone and report its antibacterial activity against various pathogenic and non-pathogenic bacterial strains.

Results and discussion

A retrosynthetic analysis of thailandamide lactone (**2**) is shown in Scheme 1. We envisioned that the target molecule could be constructed from vinyl iodide **4** and 1,3-dione-containing

[a]School of Chemical Sciences, Indian Association for the Cultivation of Science, Jadavpur, Kolkata-700032, India. E-mail: ocrkg@iacs.res.in

[b]School of Biological Sciences, Indian Association for the Cultivation of Science, Jadavpur, Kolkata-700032, India. E-mail: ritesh.pal@iacs.res.in

† Electronic supplementary information (ESI) available. CCDC 2184015 and 2184016. For ESI and crystallographic data in CIF or other electronic format see DOI: https://doi.org/10.1039/d2sc04727f

Fig. 1 Chemical structures of thailandamide family natural products.
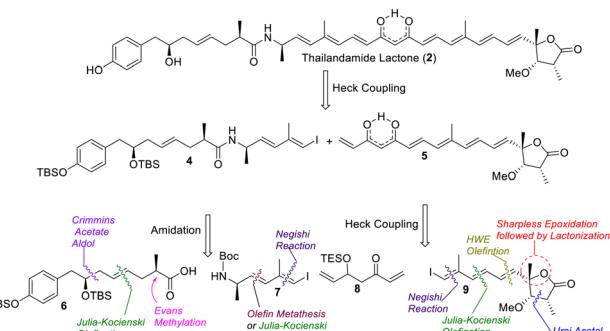
Scheme 1 Retrosynthetic analysis of thailandamide lactone (**2**).

polyene **5** adopting intermolecular Heck coupling as the key step. Vinyl iodide **4** could further be made from compounds **6** and **7** by performing amide coupling. Compound **6** could be assembled using the Crimmins acetate aldol reaction, Julia-Kocienski olefination and Evans methylation as the key steps, whereas compound **7** could be accessed utilizing cross olefin metathesis or Julia-Kocienski olefination and the Negishi reaction as the pivotal steps. On the other hand, keto-alkene **5** could be prepared from intermediates **8** and **9** using Heck coupling, whereas compound **9** could be synthesized using the Negishi reaction, Julia-Kocienski olefination, HWE olefination, Urpi acetal aldol reaction, Sharpless asymmetric epoxidation and subsequent γ-lactonization as the salient steps.

The synthesis of intermediate **6** was commenced with the known compound **11** (Scheme 2) prepared from commercially available 4-hydroxy benzaldehyde (**10**) following a literature procedure;[6] compound **11** was subjected to a Crimmins acetate aldol reaction[7] using the known auxiliary **12**[7b] in the presence of TiCl$_4$/DIPEA to obtain compound **13** as the major product in 80% yield along with its minor counterpart (dr = 4:1). The major aldol product was separated from other components using silica gel column chromatography and its structure was confirmed unambiguously using X-ray crystallographic analysis. Next, compound **13** was treated with TBSOTf/2,6-lutidine followed by NaBH$_4$ to access compound **14**, which was subjected to the Mitsunobu reaction[8] using 1-phenyl-5-thiotetrazole (**15**) in the presence of DIAD/PPh$_3$ and oxidized further using

$(NH_4)_6Mo_7O_{24} \cdot 4H_2O/H_2O_2$ in ethanol[5c] to achieve sulfone **16** in very good overall yield. Next, sulfone **16** was subjected to Julia-Kocienski olefination[5c,9] with the known aldehyde **17**[10] using KHMDS to obtain the corresponding *E*-coupled isomer as the major product along with its minor *Z*-isomer (dr = 4:1). The purified major isomer was subsequently treated with PPTS to obtain alcohol **18**, which finally was oxidized to acid **6** using Swern oxidation followed by Pinnick oxidation.[11]

The synthesis of intermediates **7** and **8** is depicted in Scheme 3. The known vinyl iodide **19**,[12] prepared from propargylic alcohol using the Negishi reaction as the key step, was subjected to the Mitsunobu reaction using 1-phenyl-5-thiotetrazole (**15**) and the resultant sulfide was oxidized using $(NH_4)_6$-$Mo_7O_{24} \cdot 4H_2O/H_2O_2$ in dioxane[13] to access sulfone **20**. Notably, the production of sulfone **20** from its corresponding sulfide was found to be much more efficient in dioxane than in the commonly used ethanol. Sulfone **20** was then reacted with the known aldehyde **21**[14] following the Julia-Kocienski olefination protocol.[9,15] Several conditions were screened for synthesizing compound **7** (Table 1) and the use of KHMDS in DME (entry-4) was found to be the best (*E/Z* = 3:1). In parallel, the cross metathesis[16] between the known alkenes **22**[17] and **23**[18] was also investigated and it was observed that HG-II produced compound **7** in 32% yield with much better selectivity (*E*:*Z* = 10:1) compared to Julia-Kocienski olefination whereas G-II and HG-I functioned ineffectively, leaving a trace amount of the desired product. However, the geometrical isomers remained inseparable at this stage. On the other hand, commercially available Weinreb amide **24** was transformed to the known compound **25** following a literature procedure[19] and subjected further to a reaction with vinyl magnesium bromide to access intermediate **8** in very good overall yield.

The synthesis of aldehyde **34** is described in Scheme 4. The known aldehyde **27** prepared from prenol (**26**) following a literature method[20] was converted to the corresponding acetal using $(MeO)_3CH/CSA$, which was subjected further to the Urpi acetal aldol reaction[5b,21] in the presence of TiCl$_4$/DIPEA/SnCl$_4$ to access compound **29** with excellent selectivity (dr = 20:1). The purified compound was then treated with LiOH·H$_2$O/H$_2$O$_2$ followed by NaOMe/MeOH to obtain compound **30** in 72% yield. The stereochemistry of asymmetric centers newly generated using Urpi acetal aldol reaction was confirmed further from an X-ray
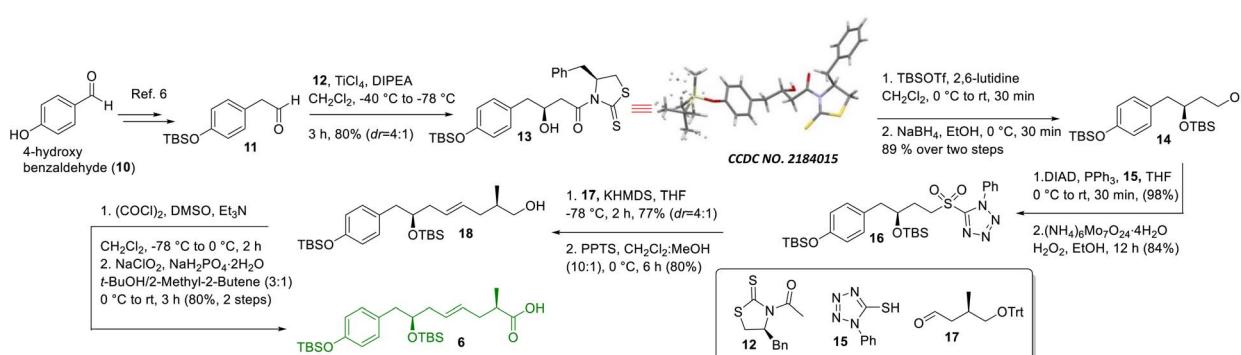

Scheme 2 Synthesis of intermediate **6**.
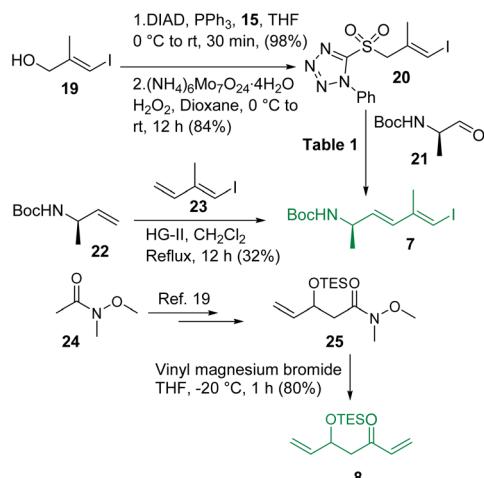
Scheme 3 Synthesis of intermediates **7** and **8**.

Table 1 Efforts to optimize Julia–Kocienki olefination

Entry	Conditions	Yield ($E:Z$)
1	NaHMDS, THF, $-78\ ^{\circ}C$	66% (1:1.2)
2	KHMDS, THF, $-78\ ^{\circ}C$	70% (1:1)
3	LiHMDS, DME, $-60\ ^{\circ}C$	62% (1.5:1)
4	KHMDS, DME, $-60\ ^{\circ}C$	68% (3:1)

crystallographic analysis of compound **31**, which was synthesized from compound **30** by performing tritylation. Next, compound **30** was reacted with BnBr/K$_2$CO$_3$ to obtain benzyl ester **32**, which was subjected to Sharpless asymmetric epoxidation[22] followed by hydrogenation to produce the corresponding epoxy acid. The stage was set for γ-lactonization.[23] The corresponding epoxy acid was treated with CSA/CH$_2$Cl$_2$ to access the 5-exo cyclized product **33** exclusively. The characteristic NOESY correlation of C$_4$–Me with C$_2$–H and C$_3$–H confirmed its structure unambiguously. We did not observe the formation of any other possible γ-lactone originating *via* 6-endo cyclization followed by concomitant acyl migration.[23] Our exhaustive efforts for achieving oxidative cleavage of the diol moiety of compound **33** using either NaIO$_4$ or NaIO$_4$/NaHCO$_3$
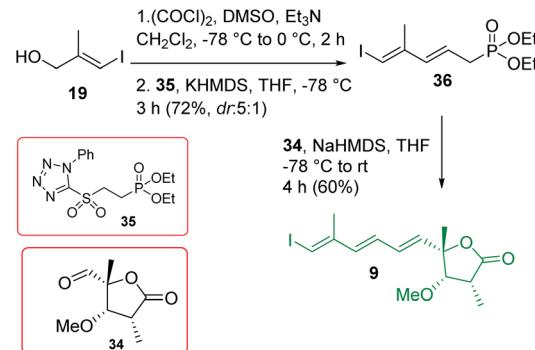
**Scheme 5** Synthesis of intermediate **9**.

did not produce aldehyde **34** in an isolable yield due to its rapid decomposition. Delightfully, silica-supported NaIO$_4$[24] provided considerable relief here, where the required aldehyde was obtained quantitatively.

The construction of compound **9** is shown in Scheme 5. Alcohol **19** was oxidized using the Swern condition and subjected to Julia-Kocienski olefination[9] with the known sulfone **35**[25] to access compound **36** as a major product (dr = 5:1). The purified major isomer was reacted further with aldehyde **34** in the presence of NaHMDS/THF following the HWE olefination protocol[25] to achieve intermediate **9** exclusively. The initially encountered isomerization problem with the α-methyl center was overcome by performing a controlled addition of NaHMDS and also by reducing the reaction time (see the optimization in Table S1 in ESI†).

The synthesis of major coupling partners (**4** and **5**) of thailandamide lactone is described in Scheme 6. Compound **7** was treated with 10% TFA/CH$_2$Cl$_2$ and the resultant Boc-deprotected amine was coupled with acid **6** to access the western segment **4** in 80% yield (over two steps). On the other hand, vinyl ketone **8** was subjected to intermolecular Heck coupling with compound **9** in the presence of Pd(OAc)$_2$/Bu$_4$NCl/Et$_3$N in DMF[26] to obtain the corresponding coupled product in complete regioselectivity, and this coupled species was further treated with CSA to obtain the corresponding β-hydroxy ketone in 77% yield after two steps. Substantial trials have been conducted to optimize its conversion to the eastern segment **5**. Most of the oxidizing agents including DMP/NaHCO$_3$ did not function properly as
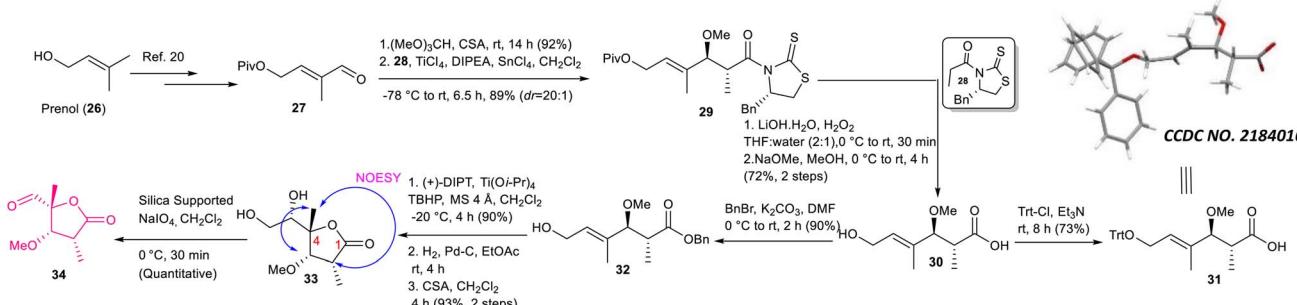
Scheme 4 Synthesis of aldehyde **34**.
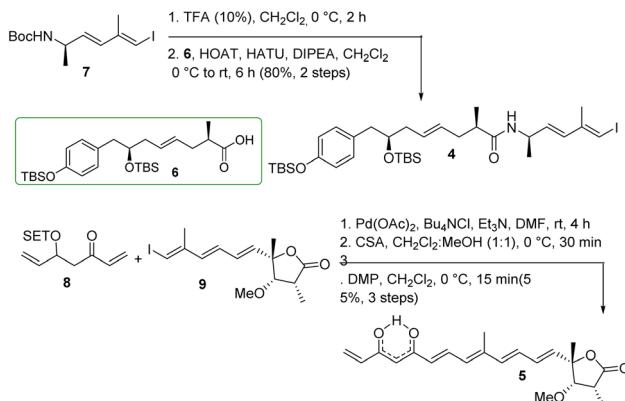
Scheme 6 Synthesis of coupling partners **4** and **5**.

their use ended up with complete decomposition of the product. However, DMP without $NaHCO_3$ produced the required product **5** in 73% yield. The appearance of a signal at a δ of 15.6 ppm in the ^1H NMR spectrum of compound **5** and two carbonyl carbons at δ 183.0, 183.9 ppm in its ^{13}C NMR spectrum clearly ascertained its existence as keto–enol tautomeric mixtures.

The completion of the total synthesis of thailandamide lactone is depicted in Scheme 7 where the stage was set for the crucial coupling between the western (**4**) and eastern (**5**) segments. Extensive efforts were made to optimize the Heck coupling (Table 2). Trials with $PdCl_2(MeCN)_2/Et_3N/HCO_2H$ in MeCN (entry-1)[27] ended up with complete decomposition of staring materials, whereas those with $Pd(PPh_3)_4/Et_3N/Bu_4NCl$ in

Scheme 7 Completion of the synthesis of thailandamide lactone (**2**).

DMF (entry-2)[27] provided the coupled product **37** in a trace amount. Use of $Pd(PPh_3)_2Cl_2/K_2CO_3/Bu_4NCl$ in DMF (entry-3), $Pd(OAc)_2/Et_3N/Bu_4NCl$ in DMF (entry-4)[27] and $Pd(OAc)_2/K_3PO_4$ in DMF (entry-5)[27c] resulted in the required product in 10%, 45% and 40% yields, respectively. A mixture of some unidentified compounds was formed along with the required compound **37** in most of the cases. Having moderate success in the transformation of compound **37** using either Pd(0) or Pd(II), we then turned our attention towards Pd(I)-catalyzed Heck coupling as it has provided excellent results in some cases.[28] Thus, [Pd(μ-I)(Pt-Bu$_3$)]$_2$ (entry-6),[28b] prepared from PdI_2/P^tBu_3 following a literature report,[28a] was then screened in the presence of DIPEA/toluene to furnish the coupled product in an improved yield (58%). Later, an equimolar mixture of $Pd(OAc)_2$ and $Pd(PPh_3)_4$ in the presence of K_3PO_4/DMF (entry-7)[28e] was tested. Delightfully, this reaction was found to proceed in a considerably cleaner manner than those with all the other tested conditions, and the coupled product **37** was obtained in 77% yield. All the reactions were performed at room temperature to reduce the rate of decomposition. A detailed NMR study unambiguously confirmed the identity of compound **37** (see the 2D spectra in ESI†). Notably the attempted synthesis of the corresponding compound requisite for an alternative Heck coupling with compound **9** was not successful—because the corresponding β-hydroxy ketone obtained from the Heck coupling between compounds **4** and **8** followed by subsequent TES ether deprotection was found to be very sensitive to various oxidizing agents including DMP. Next, compound **37** was subjected to global deprotection using HF Py to access compound **2**[29] in 89% yield. ^1H and ^{13}C NMR data (see comparison Table S2 in ESI†), optical rotation results {observed $[\alpha]_D^{28} = -43.20$ (c 0.24, methanol); reported $[\alpha]_D = -45.76$}, and HRMS, FT-IR and UV-visible spectra (see ESI†) of synthesized compound **2** were found to be in good agreement with reported data of the isolated thailandamide lactone, which unambiguously confirmed its first total synthesis.

Having thailandamide lactone in hand, we then screened its antibacterial activity against different non-pathogenic and pathogenic Gram-positive bacteria such as *Bacillus subtilis* (PY79), *Bacillus megaterium* (2G), *Staphylococcus aureus* as well as Gram-negative bacteria such as *Vibrio cholerae* (N16961), *Enteropathogenic Escherichia coli* (EPEC e2348/69), and *Escherichia coli* (MC1061)]. This screening revealed its moderate to potent antibacterial activity (Table 3). The efficacies of
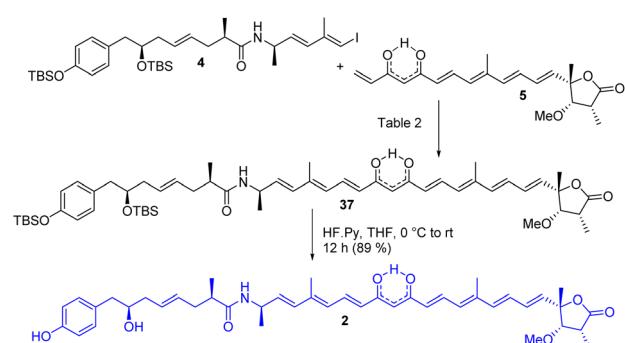
Table 2 Optimization of final Heck coupling

Entry	[Pd] (mol%)	Condition	Yield (%)
1	$PdCl_2(MeCN)_2$ (10)	Et_3N, HCO_2H, MeCN, rt, 3 h	Decomposition
2	$Pd(PPh_3)_4$ (5)	Et_3N, Bu_4NCl, DMF, rt, 6 h	Trace
3	$PdCl_2(PPh_3)_2$ (10)	K_2CO_3, Bu_4NCl, DMF, rt, 12 h	10
4	$Pd(Oac)_2$ (5)	Et_3N, Bu_4NCl, DMF, rt, 4 h	45′
5	$Pd(Oac)_2$ (5)	K_3PO_4, DMF, rt, 12 h	40
6	[Pd(μ-I)(P^{t}Bu$_3$)]$_2$ (7.5)	DIPEA, toluene, rt, 9 h	58
7	$Pd(Oac)_2$, (10) $Pd(PPh_3)_4$ (10)	K_3PO_4, DMF, rt, 18 h	77

Table 3 Antibacterial activities of thailandamide lactone

Staining type	Strains	MIC ($\mu g\ ml^{-1}$)
Gram negative	*V. cholerae* (N16961) (pathogenic)	71.3
	EPEC (e2348/69) (pathogenic)	71.3
	E. coli (MC1061)	53.5
Gram positive	*B. subtilis* (PY79)	57.0
	B. megaterium (2G)	53.5

thailandamide lactone even against Gram-negative strains were found to be promising.

Conclusions

In summary, we have developed a convergent route for the first total synthesis of a structurally challenging and labile polyketide natural product, namely thailandamide lactone, starting from the known compound **26** in 17 LLS with 8.5% overall yield. Our synthesis includes several coupling operations including two intermolecular Heck reactions. Notably, Pd(I)-based Heck coupling has been introduced for the first time, to the best of our knowledge, in the total synthesis of natural product. The possible site to couple the highly sensitive eastern and western polyene segments of thailandamide lactone was determined. The antibacterial activities of thailandamide lactone against different bacterial strains have been disclosed. Importantly, our modular strategy is expected to be amenable to thailandamide A, another member of this family as well as to structurally simplified designed analogues for further antibacterial study.

Data availability

Please see the ESI† for the data related to the manuscript.

Author contributions

R. K. G. conceived the idea, designed the hypothesis and managed overall manuscript preparation. R. R. P. planned and supervised the biological testing and assisted with manuscript preparation. H. S. and J. M. completed the total synthesis, A. K. G. was responsible for biological testing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Notes and references

1 T. Nguyen, K. Ishida, H. Jenke-Kodama, E. Dittmann, C. Gurgui, T. Hochmuth, S. Taudien, M. Platzer, C. Hertweck and J. Piel, *Nat. Biotechnol.*, 2008, **26**, 225–233.
2 K. Ishida, T. Lincke, S. Behnken and C. Hertweck, *J. Am. Chem. Soc.*, 2010, **132**, 13966–13968.
3 K. Ishida, T. Lincke and C. Hertweck, *Angew. Chem., Int. Ed.*, 2012, **51**, 5470–5474.
4 Y. Wu and M. R. Seyedsayamdost, *Biochemistry*, 2018, **57**, 4247–4251.
5 (*a*) S. Saha, D. Paul and R. K. Goswami, *Chem. Sci.*, 2020, **11**, 11259–11265; (*b*) T. K. Kuilya and R. K. Goswami, *Org. Lett.*, 2017, **19**, 2366–2369; (*c*) S. Das, D. Paul and R. K. Goswami, *Org. Lett.*, 2016, **18**, 1908–1911; (*d*) S. Guchhait, S. Chatterjee, R. S. Ampapathi and R. K. Goswami, *J. Org. Chem.*, 2017, **82**, 2414–2435; (*e*) D. Saha, G. H. Mandal and R. K. Goswami, *J. Org. Chem.*, 2021, **86**, 10006–10022.
6 A. Ruiz-Olalla, M. A. Wurdemann, M. J. Wanner, S. Ingemann, J. H. van Maarseveen and H. Hiemstra, *J. Org. Chem.*, 2015, **80**, 5125–5132.
7 (*a*) M. T. Crimmins, B. W. King, E. A. Tabet and K. Chaudhary, *J. Org. Chem.*, 2001, **66**, 894–902; (*b*) M. T. Crimmins, *Org. Lett.*, 2007, **9**, 149–152.
8 O. Mitsunobu, *Synthesis*, 1981, **1**, 1–28.
9 P. Liu and E. N. Jacobsen, *J. Am. Chem. Soc.*, 2001, **123**, 10772–10773.
10 A.-F. Salit, C. Meyer and J. Cossy, *Synlett*, 2007, **6**, 934–938.
11 P. Li, J. Li, F. Arikan, W. Ahlbrecht, M. Dieckmann and D. Menche, *J. Am. Chem. Soc.*, 2009, **131**, 11678–11679.
12 C. L. Rand, D. E. Van Horn, M. W. Moore and E. Negishi, *J. Org. Chem.*, 1981, **46**, 4093–4096.
13 T. P. Ahern, H. O. Fong, R. F. Langler and P. M. Mason, *Can. J. Chem.*, 1980, **58**, 878–883.
14 H. Yao and K. Yamamoto, *Chem.–Asian J.*, 2012, **7**, 1542–1545.
15 T. J. Harrison, S. Ho and J. L. Leighton, *J. Am. Chem. Soc.*, 2011, **133**, 7308–7311.
16 (*a*) D. Saha, S. Guchhait and R. K. Goswami, *Org. Lett.*, 2020, **22**, 745–749; (*b*) K. C. Nicolaou, P. G. Bulger and D. Sarlah, *Angew. Chem., Int. Ed.*, 2005, **44**, 4442–4489; (*c*) S. B. Garber, J. S. Kingsbury, B. L. Gray and A. H. Hoveyda, *J. Am. Chem. Soc.*, 2000, **122**, 8168–8179; (*d*) Y. Zhang, M. Dlugosch, M. Jubermann, M. G. Banwell and S. J. Ward, *J. Org. Chem.*, 2015, **80**, 4828–4833.
17 J. Ryan, M. Saiuciulis, A. Gomm, B. Maciá, E. O'Reilly and V. Caprio, *J. Am. Chem. Soc.*, 2016, **138**, 15798–15800.
18 S. Ma and E.-i. Negishi, *J. Org. Chem.*, 1997, **62**, 784–785.
19 J. L. Tyler, A. Noble and V. K. Aggarwal, *Angew. Chem., Int. Ed.*, 2021, **60**, 11824–11829.
20 J. A. González-Delgado, J. F. Arteaga, M. M. Herrador and A. F. Barrero, *Org. Biomol. Chem.*, 2013, **11**, 5404–5408.
21 A. Cosp, P. Romea, P. Talavera, F. Urpí, J. Vilarrasa, M. Font-Bardia and X. Solans, *Org. Lett.*, 2001, **3**, 615–617.
22 T. Katsuki and K. B. Sharpless, *J. Am. Chem. Soc.*, 1980, **102**, 5974–5976.

23 J. S. Clark, C. A. Baxter, A. G. Dossetter, S. Poigny, J. L. Castro and W. G. Whittingham, *J. Org. Chem.*, 2008, **73**, 1040–1055.

24 Y. L. Zhong and T. K. M. Shing, *J. Org. Chem.*, 1997, **62**, 2622–2624.

25 N. Cichowicz and P. Nagorny, *Org. Lett.*, 2012, **14**, 1058–1061.

26 R. F. Heck, *Org. React.*, 1982, **27**, 345–390.

27 (*a*) I. P. Beletskaya and A. V. Cheprakov, *Chem. Rev.*, 2000, **100**, 3009–3066; (*b*) A. de Meijere and F. E. Meyer, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 2379–2411; (*c*) Q. Yao, E. P. Kinney and Z. Yang, *J. Org. Chem.*, 2003, **68**, 7528–7531.

28 (*a*) R. Vilar, D. M. P. Mingos and C. J. Cardin, *J. Chem. Soc., Dalton Trans.*, 1996, **23**, 4313–4314; (*b*) F. Schoenebeck, T. Sperger and C. Stirner, *Synthesis*, 2016, **49**, 115–120; (*c*) M. Aufiero, T. Sperger, A. S. Tsang and F. Schoenebeck, *Angew. Chem., Int. Ed.*, 2015, **54**, 10322–10326; (*d*) F. Proutiere, M. Aufiero and F. Schoenebeck, *J. Am. Chem. Soc.*, 2012, **134**, 606–612; (*e*) G. Magnin, J. Clifton and F. Schoenebeck, *Angew. Chem., Int. Ed.*, 2019, **58**, 10179–10183; (*f*) C. Fricke, T. Sperger, M. Mendel and F. Schoenebeck, *Angew. Chem., Int. Ed.*, 2021, **60**, 3355–3366.

29 Compound **2** was found highly sensitive to light as well as quite unstable. It decomposed to a mixture of unidentified compounds. The colour changed from yellow to dark red. It was observed that use of Silica gel for purification and glass vessel for storing was not suitable as it underwent faster decomposition. Notably, the major part of compound stuck to reverse phase C18 column (Xbridge RP18) during purification.