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Total Synthesis of Dehaloperophoramidine using a highly diastereoselective Hosomi-Sakurai reaction

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The synthesis of dehaloperophoramidine, a non-halogenated derivative of the marine natural product perophoramidine, and its biological activity towards HCT116, HT29 and LoVo colorectal carcinoma cells is reported. A [3,3]-Claisen rearrangement and an epoxide opening/allylsilylation reaction installed the contiguous all-carbon quaternary stereocentres with the required relative stereochemistry.

The natural product (+)-perophoramidine (**1**) was isolated by Ireland from the marine ascidian *Perophora namei*.¹ The authors also reported the structure of dehaloperophoramidine (**2**), which they obtained *via* transfer hydrogenation of (+)-**1**. These interesting alkaloids are structurally related to a more complex family of natural products that includes Communesin F (**3**).² Targets, including **1**, **2** and **3**, that possess contiguous all-carbon quaternary stereocentres present a significant synthetic challenge.³ The presence of this structural unit and the anticancer activity reported for **1** has led to several total and formal syntheses.⁴ Previous approaches to **2** have involved a spirocyclisation of a 2-thiotryptamine analogue⁵, a dearomatising arylation of a quinoline⁶ and an efficient synthesis from a commercially available indigo dye.⁷

In work aimed at the synthesis of the communesins, we have explored a [3,3]-Claisen approach to establish the required C7 stereocentre in **3** (Figure 1).⁸ In the first part of this new report we describe a significant extension of this work resulting in the large scale synthesis (62 grams) and resolution of a novel ketone using our [3,3]-Claisen rearrangement method (Scheme 1). This highly efficient sequence, that does not require chromatographic

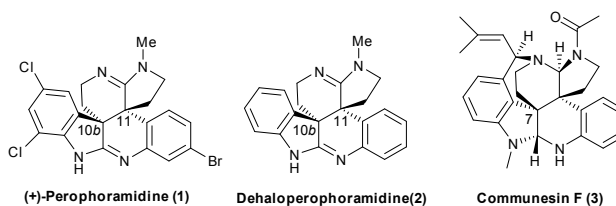
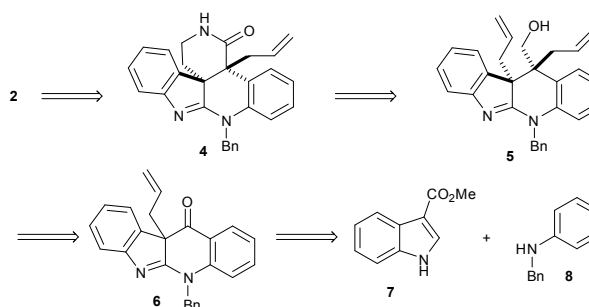


Fig. 1 The structures of (+)-perophoramidine (**1**), (+)-dehaloperophoramidine (**2**) and communesin F (**3**).

purification in any of the first five steps, enables access to enantio-enriched ketones. Whilst we view these ketones as flexible starting points for the synthesis of a number of optically pure complex ring systems, here we report conversion of racemic material to **2**.

Our retrosynthetic analysis of **2** (Scheme 1) identified lactam **4** as the cornerstone of the approach. Lactam **4** was viewed as accessible from diallyl-substituted alcohol **5**, which contains both the required C-10b and C-11 stereocentres. A key challenge in this part of the synthesis was the need to differentiate between the two allyl groups in **5**. This was achieved using two different selective iodocyclisation protocols. Whilst this transformation is preceded in simpler systems,⁹ it has been used sparingly in the synthesis of more complex alkaloid-based structures.¹⁰ Alcohol **5** could be prepared from allyl-ketone **6**, which itself could be constructed from commercially available **7** and **8**.



Scheme 1 Retrosynthetic analysis of **2** starting from commercially available **7** and **8**.

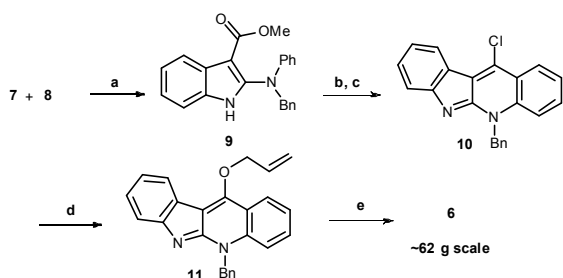
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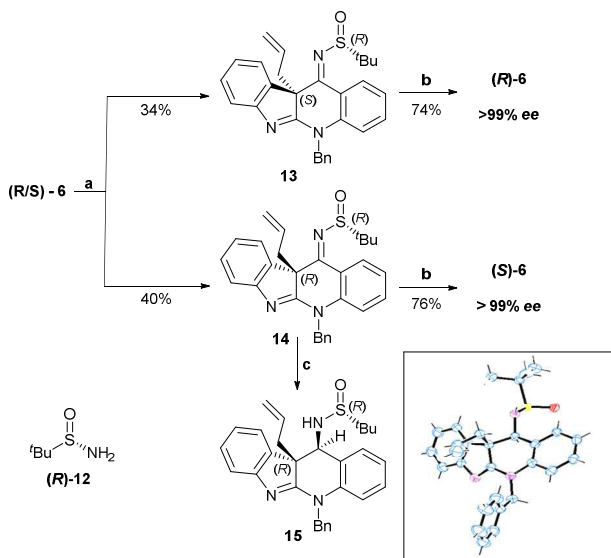
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Scheme 2 Construction of key intermediate **6**: a) NCS, DMP, DCM, 0 °C, 2 hrs; then TCA, 0 °C – rt, 2 hrs, 93%; b) Ph₂O, reflux, 2.5 hrs, 79%; c) POCl₃, reflux, 95%; d) Na (3 eq.), allyl alcohol (5.7 eq.), rt, 18 hrs, 92%; e) PhMe, reflux, 1.5 hrs, 88%.

The key challenge in the conversion of **6** to **5** is the incorporation of the second contiguous all carbon stereogenic centre with the required relative stereochemistry. Conversion of the ketone in **6** to the corresponding epoxide was planned. Subsequent Lewis acid catalysed epoxide opening followed by trapping with allylsilane was predicted to lead to **5** with the approach of the allyl nucleophile occurring from the opposite face to the allyl group that is already present. This reaction, which we refer to as a modified Hosomi-Sakurai reaction¹¹, has been used in natural product synthesis,¹² but to the best of our knowledge has not been used in the construction of contiguous all-carbon quaternary centres. Alternative approaches to lactam **4** involving the early development of the allyl group in **6** or alkylation of a C11-based ester were explored but were either ultimately unsuccessful or led to significantly longer reaction sequences (data not shown).

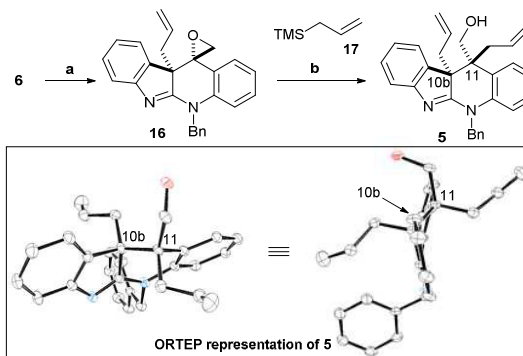
The C10b stereocentre in **6** was installed in 5 steps from **7** and **8** as described in Scheme 2. After coupling of **7** and **8** to give **9**, the tetracyclic system was formed using a high temperature electrophilic aromatic substitution reaction (see Scheme S1¹³ for additional studies). The cyclisation was scalable up to 100 g with no detrimental effect on the yield. Functional group manipulations then enabled the preparation, *via* **10**, of the [3,3]-Claisen rearrangement substrate **11**. Highly efficient conversion of **11** to the required novel ketone **6** occurred on heating in toluene for 1.5 hours in 88% yield. This reaction was robust and scalable with 62 g of **6** being prepared in a single batch and without the need for purification by column chromatography at any stage. Ketone **6** could be resolved by treatment with (*R*)-*tert*butanesulfinamide (**12**) to generate the readily separable diastereomeric imines **13** and **14** (Scheme 3). This approach was inspired by the reported resolution of a ketone-containing intermediate *en route* to epoxidine.¹⁴ Hydrolysis of imines **13** and **14** with 12M HCl in MeOH gave (*R*)-**6** and (*S*)-**6** respectively in > 99% *ee* after recrystallisation (confirmed by chiral HPLC analysis¹³). The absolute configuration of the intermediate imines **13** and **14** were assigned following X-ray crystallographic analysis of **15**¹⁵ which was obtained following diastereoselective reduction of **14** with NaBH₄ (Scheme 3). The X-ray analysis indicated that the absolute configuration of the C10b stereogenic centre in **15** (and hence in **14**) was (*R*). Hydrolysis of imine **14** therefore provided the (*S*)-enantiomer of **6** (and **13** gave



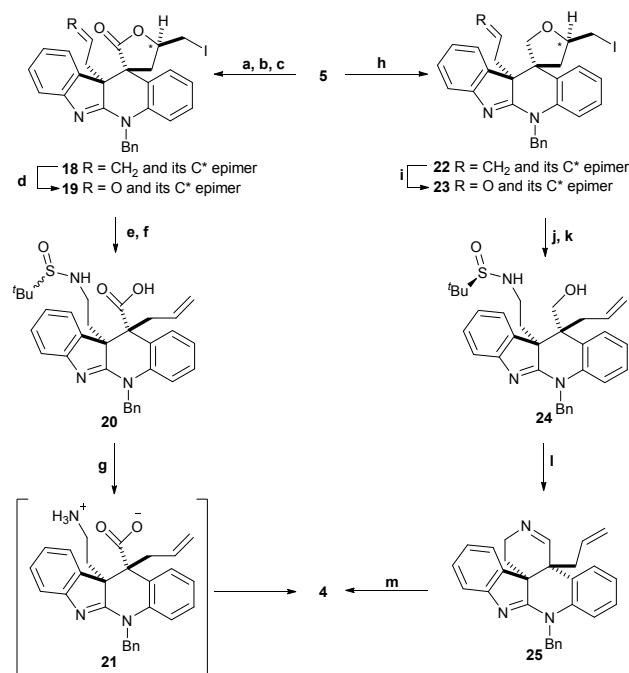
Scheme 3 Obtaining the (*R*) and (*S*) enantiomers of ketone **6** from their respective imines **13** and **14**. The ORTEP representation of **15** is also shown and was used to infer the absolute configuration of **14** and (*S*)-**6** (and hence **13** and (*R*)-**6**). Reagents and conditions: a) *R*-**12** (10 eq.), Ti(OEt)₄ (15 eq.), toluene, 85 °C, 9 hrs b) 12M HCl, MeOH, rt, 0.5 hr; c) NaBH₄ (2.5 eq.), MeOH, rt, 0.5 hr, 92%.

(*R*)-**6**). Whilst (*R*)-**6** and (*S*)-**6** have been prepared, their conversion to enantio-enriched alternative complex ring systems will be reported in the future. Here we decided to continue the development of a route to **2** using the much larger quantities of (\pm)-**6** available to us.

With the novel ketone **6** in hand, the ketone functionality was converted to the corresponding epoxide using ClCH₂I¹⁶ (see Scheme S2 for the stereochemical assignment of **16**).¹³ Allylsilylation of **16** under Lewis acidic conditions with allylTMS (**17**) in a modified Hosomi-Sakurai reaction¹¹ gave the desired alcohol **5** as a single diastereoisomer (as judged by ¹H NMR analysis of the crude reaction mixture). This reaction proved highly robust and scalable with *ca.* 42 g of **16** being reproducibly converted to 38 g of **5**. X-ray crystallographic analysis of **5** confirmed the required *anti*-relationship of the two allyl substituents at C-10b and C-11 (Schemes 4 and S3).¹³



Scheme 4 Construction of all-carbon quaternary stereocentres. The ORTEP representation of **5** is also shown.¹⁵ Reagents and conditions: a) ClCH₂I (1.1 eq.), MeLiBr (2.2 M solution in Et₂O, 1.5 eq.), THF, -78 °C, 2 hrs; b) AllylTMS (**17**) (2.5 eq.), TiCl₄ (4 eq.), DCM, -78 °C, 78% (2 steps).



The challenge of selectively functionalising the two allyl groups in **5** now had to be overcome. A two-step oxidation of **5**, followed by regioselective iodolactonisation gave **18** and its epimer at the indicated carbon as an inconsequential mixture of diastereoisomers (Scheme 5, d.r. 3:1). The relative configuration of the newly formed stereocentre in the major isomer **18** was determined by nOe analysis (Scheme S4 and Figures S2-S4).¹³ Oxidative cleavage on treatment with catalytic OsO₄ and NMO followed by *in situ* reaction with Ph(OAc)₂ gave **19** and its epimer (Schemes 5 and S5).¹³ After incorporation of the required nitrogen by reductive amination using (±)-**12**¹⁷ (Scheme 3), retro-iodolactonisation to give the diastereomeric mixture **20**, acid-mediated deprotection and subsequent cyclisation of **21** with HBTU and DIPEA, lactam **4** was obtained. However, cyclisation of **20** to **4** proved irreproducible (for further discussion see Schemes S6-S8 and Figure S5).¹³ An alternative route to **4** was investigated in an attempt to circumvent the reproducibility issue. Di-allyl alcohol **5** underwent a regio- and highly diastereo-selective iodoetherification to give **22** (and its epimer, d.r. 11:1, Figure S6)¹³ in the presence of iodine under basic conditions. A one-pot oxidative cleavage of the alkene gave **23** (and its epimer) which underwent successful reductive amination with (R)-**12** followed by a retro-iodoetherification in the presence of Zn

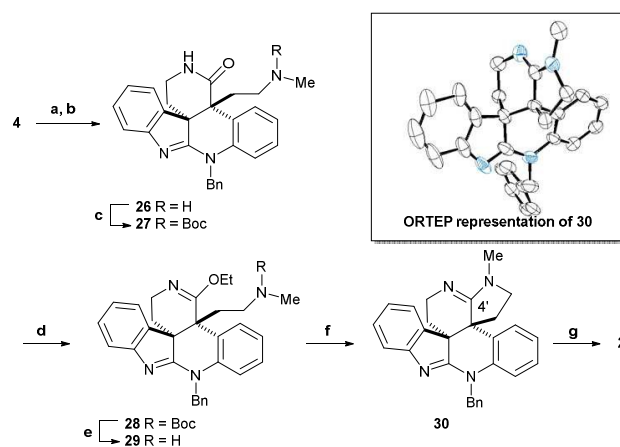
to generate the diastereomeric mixture **24**. Oxidation of **24** with the Dess-Martin periodinane¹⁸ gave **25** in a reasonable yield (52%) for this relatively complex process which also involves acid-mediated deprotection of the sulfonamide. Interestingly, **25** underwent oxidation with NaClO₂ to give the required lactam **4**. This transformation was inspired by a report by Tomioka *et al.* on an unrelated system.¹⁹ The new approach was robust and overall reduced the length of the reaction sequence to **4**.

Having successfully synthesised the desired lactam **4**, its conversion to **2** was completed (Scheme 6). The remaining alkene in lactam **4** was oxidatively cleaved and the resulting aldehyde reductively aminated using MeNH₂·HCl under basic conditions to give **26** (Scheme 6). Boc protection of **26** gave **27** which underwent alkylation with Meerwein's reagent²⁰ to give **28**.¹³ Deprotection of **28** with trifluoroacetic acid gave **29** which cyclised on refluxing in toluene to give *N*-benzyl-dehaloperophoramidine (**30**) in excellent yield. X-ray crystallographic analysis of **30** confirmed the successful formation of the C-4' amidine motif.¹⁵ The synthesis of **2** was completed by *N*-benzyl deprotection of **30** via a single electron transfer process with a freshly prepared solution of sodium naphthalene.²¹

Synthetic **2** was converted to the corresponding TFA salt¹³ and compared to an authentic sample of **2.TFA** (Figures S7 - S9).¹³ A doping experiment with authentic **2.TFA** and synthetic **2.TFA** confirmed that the desired compound had been successfully prepared (see Figures S8A and S8B for a comparison of a selected region of the ¹H NMR analysis carried out in CD₃OD). Superimposition of the ¹H NMR of the doped sample with the ¹H NMR of authentic **2.TFA** alone displayed enhancement of the signals corresponding to the product whilst an impurity from authentic **2.TFA** remained unchanged (Figure S8C for selected aryl and Figure S8D for alkyl signals)¹³

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Scheme 6 Completion of the synthesis of (±)-**2**. Reagents and conditions: a) OsO₄ (3.2 mol%), NMO (1.5 eq.), THF/H₂O, rt, 16 hrs, then Ph(OAc)₂ 1.5 hrs, 62% (over 2 steps from **25**); b) MeNH₂·HCl (2.5 eq.), NaOAc (2.5 eq.), MeOH, rt, 16 hrs, then NaBH₄ (3 eq.), rt, 0.5 hrs; c) (Boc)₂O (1.5 eq.), TEA (1.5 eq.), rt, 1 hr, 44% (2 steps); d) Meerwein's reagent (1 M solution in DCM, 8 eq.), DIPEA (8 eq.), DCM, 0 °C – rt, 2 hrs, 72%; e) 5% TFA, DCM, 0 °C, 0.5 hrs; f) DIPEA (2 eq.), toluene, reflux, 16 hrs, 83% (over 2 steps); g) Na/naphthalene (1 M solution), THF, 0 °C – rt, 2 hrs, 50%.

When reanalysed in CDCl_3 , our sample of **2**.TFA gave chemical shifts analogous to those previously reported (Table S2).¹³ Comparison of the ^1H NMR analysis of our synthetic free base **2** in CDCl_3 was also consistent with the previous literature (Tables S3).¹³

Preliminary biological activity associated with **1** has been reported by Ireland *et al.*,¹ however, no information on the activity of **2** is available. Therefore our sample of **2** was tested against the same cancer cell line (HCT116) used to assess the activity of **1** (Figure 2). Estimation of an IC_{50} inhibitory concentration showed that **2** killed HCT116 cells with similar potency to **1**, suggesting that the halogens present in **1** are not essential for biological activity. Analogous studies using the colorectal cancer lines HT29 and LoVo showed that both were more sensitive to **2** than the HCT116 cells. Attempts to test the synthetic precursor *N*-benzyldehaloperophoramidine (**30**) proved difficult due to its insolubility at the concentrations used in these assays.

In conclusion, a synthesis of dehaloperophoramidine (**2**) from commercially available starting materials has been achieved. A highly efficient [3,3]-Claisen rearrangement to give novel ketone **6** was followed by a diastereoselective epoxide opening/allylsilylation (modified Hosomi-Sakurai) reaction. In combination these reaction enabled construction of the required contiguous all carbon quaternary stereocentres. Detailed spectroscopic comparison with an authentic sample of **2** confirmed that it was identical to our synthetic TFA salt of **2**. Preliminary biological activity associated with **2** has also been reported for the first time.

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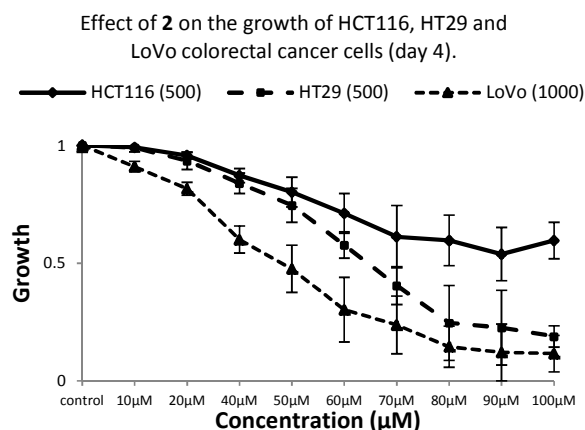


Fig. 2 Dose-response curves for the effect of our synthetic sample of **2** on the growth of three colorectal cancer cell lines. Growth data (relative to untreated controls but containing equimolar concentrations of DMSO carrier) are shown for HCT116 (solid line), HT29 (dashed line) and LoVo cells (dotted line), assessed by SRB assay four days after treatment. The number of cells plated per well at the beginning of the experiment is shown in parentheses. IC_{50} values of 60–70 μM (HCT116), 60 μM (HT29) and 50 μM (LoVo) were calculated using Graphpad Prism software and are equivalent to the IC_{50} of 60 μM reported for compound **1** by Ireland *et al.*¹

Notes and references

- S. M. Verbitski, C. L. Mayne, R. A. Davis, G. P. Concepcion, C. M. Ireland, *J. Org. Chem.*, 2002, **67**, 7124.
- For selected reviews, see a) B. M. Trost, M. Osipov, *Chemistry*, 2015, **21**, 16318; b) P. Siengalewicz, T. Gaich, J. Mulzer, *Angew. Chem. Int. Ed.*, 2008, **47**, 8170. For total syntheses of Communesins A, B and F see c) J. Yang, H. Wu, L. Shen, Y. Qin, *J. Am. Chem. Soc.*, 2007, **129**, 13794; d) J. Yang, H. Wu, L. Shen, Y. Qin, *Synfacts*, 2008, 226; e) P. Liu, J. H. Seo, S. M. Weinreb, *Angew. Chem. Int. Ed.*, 2010, **49**, 2000; f) Z. Zuo, W. Xie, D. Ma, *J. Am. Chem. Soc.*, 2010, **132**, 13226; g) Z. Zuo, D. Ma, *Angew. Chem. Int. Ed.*, 2011, **50**, 12008; h) J. Belmar, R. L. Funk, *J. Am. Chem. Soc.*, 2012, **134**, 16941.
- E. Peterson, L. E. Overman, *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 11943.
- For total and formal syntheses of perophoramidine see a) J. R. Fuchs, R. L. Funk, *J. Am. Chem. Soc.*, 2004, **126**, 5068; b) H. Wu, F. Xue, X. Xiao, Y. Qin, *J. Am. Chem. Soc.*, 2010, **132**, 14052; c) H. Zhang, L. Hong, H. Kang, R. Wang, *J. Am. Chem. Soc.*, 2013, **135**, 14098; d) S.-J. Han, F. Vogt, J. A. May, S. Krishnan, M. Gatti, S. C. Virgil, B. M. Stoltz, *J. Org. Chem.*, 2014, **80**, 528; e) S.-J. Han, F. Vogt, S. Krishnan, J. A. May, M. Gatti, S. C. Virgil, B. M. Stoltz, *Org. Lett.*, 2014, **16**, 3316; f) B. M. Trost, M. Osipov, S. Krüger, Y. Zhang, *Chem. Sci.*, 2015, **6**, 349.
- J. D. Ranier, *Angew. Chem. Int. Ed.*, 2006, **45**, 4317.
- T. Ishida, H. Ikota, K. Kurahashi, C. Tsukano, Y. Takemoto, *Angew. Chem. Int. Ed.*, 2013, **52**, 10204.
- K. Popov, A. Hoang, P. Somfai, *Angew. Chemie. Int. Ed.*, 2016, **55**, 1801.
- N. Voüte, D. Philp, A. M. Z. Slawin, N. J. Westwood, *Org. Biomol. Chem.*, 2010, **8**, 442
- C. V. Ramana, R. Murali, K. Ravikumar, M. Nagarajan, *J. Chem. Res (S)*, 1996, **5**, 226
- For a recent example of a regioselective iodoetherification in a total synthesis, see Y. Li, S. Zhu, J. Li, A. Li, *J. Am. Chem. Soc.*, 2016, **138**, 3982.
- A. Hosomi, H. Sakurai, *Tetrahedron Lett.* 1976, **17**, 1295.
- S. V. Pansare, K. G. Kulkarni, *RSC Adv.*, 2013, **3**, 19127.
- See electronic supporting information (ESI).
- C. Dallanoce, L. Rizzi, L. Pucci, C. Gotti, F. Clementi and C. De Micheli, *Chirality*, 2012, **24**, 543.
- CCDC 1486344 (**15**), CCDC 1478153 (**516**), CCDC 1478152 (**5**) and CCDC 1478154 (**30**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif
- K. M. Sadhu, D. S. Matteson, *Tetrahedron Lett.*, 1986, **27**, 795.
- a) J. A. Ellman, T. D. Owens, T. P. Tang, *Acc. Chem. Res.*, 2002, **35**, 984; b) M. T. Robak, M. A. Herbage, J. A. Ellman, *Chem. Rev.*, 2010, 3600.
- D. B. Dess, J. C. Martin, *J. Org. Chem.* 1983, **48**, 4155.
- a) M. A. Mohamed, K. Yamada, K. Tomioka, *Tetrahedron Lett.*, 2009, **50**, 3436; b) K. Yamada, Y. Mogi, M. A. Mohamed, K. Takasu, K. Tomioka, *Org. Lett.*, 2012, **14**, 5868.
- H. Meerwein, G. Hinz, P. Hofmann, E. Kroning, E. Pfeil, *J. für Prakt. Chemie*, 1937, **147**, 257.
- S. C. Bergmeier, P. P. Seth, *Tetrahedron Lett.*, 1999, **40**, 6181.