

Microalgae lipids as a feedstock for the production of benzene

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A two-step one-pot synthesis of benzene from the five-fold unsaturated fatty acid eicosapentaenoic acid (EPA), a component of microalgae oils, is presented. By a sequence of olefin metathesis and the catalytic dehydrogenation of the resulting 1,4-cyclohexadiene, two equivalents of benzene are effectively formed per EPA substrate molecule. As the only major by-products, 5-octenoic acid and 5-decenedioic acid are formed. Performing the dehydrogenation step under hydrogen pressure results in the formation of their saturated analogues, sebacic acid and octanoic acid, both desirable products, while the simultaneous dehydrogenation step to benzene is not hampered.

Introduction

Aromatics are essential key compounds of the chemical industry. In particular, benzene is the starting material for the production of numerous agrochemical and pharmaceutical actives, dyes and other chemicals. In view of the finite nature of petrochemical sources and the environmental impact of fossil fuel recovery, schemes that provide access to aromatics from renewable sources are desirable in the long term.

In principle, aromatics occur in large amounts in lignin. However, lignin is a complex mixture containing numerous oxygenated substituted benzene motifs linked to a high molar mass biopolymer. Efforts to produce single clean aromatic compounds by breaking down lignin efficiently have not been successful to date.^{1,2} Thus, lignin is used as a low value fuel for the very largest part.³ Possible schemes for the efficient generation of benzene utilizing suitable renewable molecules are lacking.

We now report on the catalytic generation of benzene from eicosapentaenoic acid (EPA). EPA is contained in substantial amounts in various microalgae or can also be produced by yeasts.⁴⁻⁶ While the limited achievable cell densities are currently a bottleneck for their production, microalgae offer the benefit of not competing with food production for arable land and fresh water, and they reproduce very rapidly to yield high oil content biomass.⁷⁻¹³

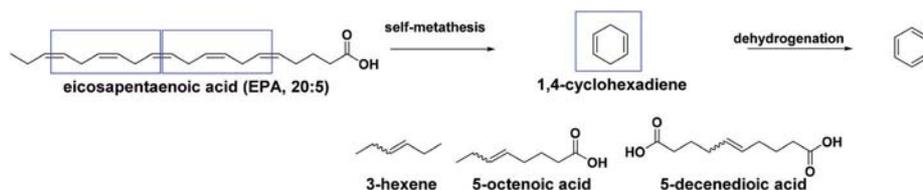
Results and discussion

Towards this aim, we pursued ring closure by olefin metathesis with subsequent dehydrogenation to yield benzene (Scheme 1). For this concept, it is notable that olefin metathesis by ruthenium alkylidenes has been shown to be compatible with fatty acid substrates, plant oils and other renewable feedstocks.¹⁴⁻¹⁸ Also, the catalytic dehydrogenation of six-membered cyclic olefins to benzene, driven by the stability of the aromatic systems, is a proven reaction.^{19,20}

Olefin metathesis usually converts the substrates into an equilibrium mixture of all possible products accessible by the formal cleavage of the double bonds and recombination of the methylene fragments. For multiple unsaturated substrates like EPA, a large range of linear and cyclic products are conceivable. Advantageously, 1,4-cyclohexadiene is a thermodynamic sink.²¹⁻²³ This not only favours the formation of this desired product, but ideally also strongly reduces the number of further products formed, namely 5-octenoic acid, 5-decenedioic acid and 3-hexene which are the result of net EPA chain-end homo-metathesis (Scheme 1).

Towards the aim of an efficient self-metathesis reaction of eicosapentaenoic acid, we screened a range of commercial metathesis catalysts (Fig. 1).

The metathesis reactions were run until no change in the composition of the reaction mixture occurred anymore, as observed by GC. These times appeared to vary from 4 hours, which the Grubbs 2nd generation catalyst required (Table 1, entry 2), to 15 minutes, when Hoveyda-Grubbs 2 achieved its maximum conversion (Table 1, entry 5). In all cases, the productivity was high; the lowest conversion achieved reached 93% with the Grubbs 1st generation catalyst (Table 1, entry 1). The selectivity towards 1,4-cyclohexadiene was however, not always complete. In most cases this appeared to be around 70



Scheme 1 Self-metathesis of eicosapentaenoic acid, and a possible subsequent homo-metathesis of the produced 5-octenoate to produce 3-hexene and 5-decenedioate, and two equivalents of benzene after dehydrogenation.

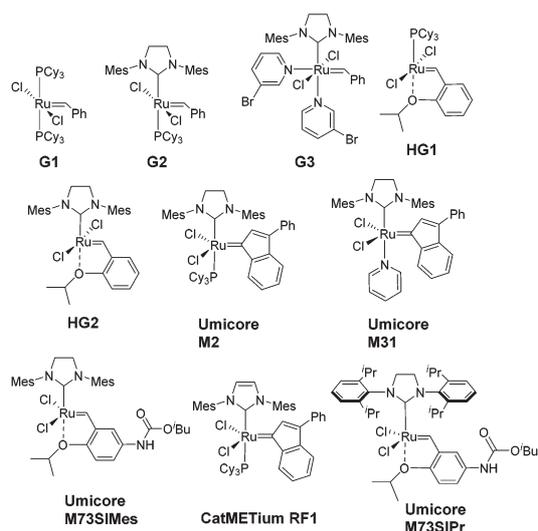


Fig. 1 Metathesis catalysts employed in the screening of eicosapentaenoic acid metathesis.

to 80%, where catMETium was much lower (55%, Table 1, entry 6) and Hoveyda-Grubbs 2 (100%, Table 1, entry 5) provided the highest selectivity. Overall, 5-octenoate and 5-decenedioic acid were the main remaining additional products. The 5-decenedioic acid results from 5-octenoic self-metathesis or direct EPA chain metathesis. These products are observed as a mixture of both in their thermodynamic equilibrium from the metathesis, about 50% of 5-octenoic acid and 25% 5-decenedioic acid.

The remaining 3-hexene was not observed due to the overlap with the solvent signal (Fig. S2, ESI[†]). In only a few cases (entries 1, 2 and 4, Table 1) the metathesis was incomplete and multi-unsaturated linear products were observed. All in all, the Hoveyda-Grubbs 2nd generation catalyst appears to be the most suitable catalyst for the self-metathesis of EPA, achieving a maximum conversion and selectivity for 1,4-cyclohexadiene.

For the dehydrogenation, different solid-supported Pd catalysts were studied, initially employing neat 1,4-cyclohexadiene as a substrate. This showed the presence of a solvent to be beneficial to perform the dehydrogenation efficiently. A hydrogen acceptor however, was not required although hydrogen is expelled from 1,4-cyclohexadiene to form benzene (ESI,† *vide infra*). Complete conversion to benzene was observed in these experiments under suitable reaction conditions.

Based on these, in view of a straightforward one-pot procedure, olefin metathesis was carried out in the presence of a solvent, and without any further purification, a solid dehydrogenation catalyst was added and the reaction mixture was heated to optimum dehydrogenation conditions (Table 2). It could be expected that the non-cyclic mono-unsaturated molecules (5-octenoate, or 5-decenedioate) present in the post-metathesis mixture of eicosapentaenoic acid might serve as a hydrogen acceptor.^{19,20,24,25} This was however, not observed to a large extent; only a small portion of octenoate and decenedioic acid was found at most, whereas the major part of the unsaturated linear molecules was unaffected (apart from some double bond isomerization; Fig. S6, ESI[†]).

Table 1 Productivity of several metathesis catalysts in the self-metathesis of EPA

Entry	Metathesis cat. (Fig. 1)	Time (min)	Conv. ^a (%)	1,4-CHD select. ^a (%)	Main (by) product ^a
1	G1	60	93	70	Undecadienoate ^b
2	G2	240	100	93	Multiple unsat. ^b
3	G3	15	100	78	5-Octenoate
4	HG1	60	100	94	Undecadienoate ^b
5	HG2	15	100	100	5-Octenoate
6	CatMETium RF1	75	99	55	5-Octenoate
7	Umicore M2	105	99	78	5-Octenoate
8	Umicore M31	75	100	72	5-Octenoate
9	Umicore M73 SIPr	75	100	91	5-Octenoate
10	Umicore M73 SIMes	105	99	84	5-Octenoate

Conditions: Eicosapentaenoic acid (5 mmol), metathesis catalyst (0.005 mmol, 0.1 mol%), neat, 45 °C. ^a Determined *via* GC analysis. Conversion is determined on the EPA consumption. The selectivity was determined with respect to the maximum theoretical amount (2 equiv. of 1,4-cyclohexadiene per EPA starting material). ^b Incomplete self-metathesis product.

Table 2 Results of one-pot metathesis and the dehydrogenation of eicosapentaenoic acid to benzene

Entry	Dehydrogenation catalyst (loading)	Metathesis conv. and select. for 1,4-CHD ^a	Conv. of 1,4-CHD ^a (%)	Overall benzene select. ^{a,b} (%)
1 ^{b,c}	Pd/C (1 mol%)	100	100	75
2 ^b	Pd/C (0.1 mol%)	100	99	73
3 ^c	Pd/C (1 mol%)	99	99	87
4	Pd/C (1 mol%)	99	99	76
5	Pd(OAc) ₂ (1 mol%)	99	99	67
6	Pd/Al ₂ O ₃ (0.1 mol%)	99	99	66
7 ^{c,d}	Pd/C (1 mol%)	38	38	28

Conditions: HG2 (0.005 mmol, 0.1 mol%), 10 mL toluene solvent, 15 min at 45 °C, then addition of the supported Pd catalyst and 110 °C for 20 h. ^a Analysed by GC, the conversion of the metathesis was determined with respect to the EPA consumption. The selectivity of 1,4-cyclohexadiene and benzene was determined with respect to the maximum theoretical amount of 2 equiv. 1,4-cyclohexadiene from EPA. ^b 0.2 equiv. AMS (anthraquinone-2-sulfonic acid sodium salt) added as a hydrogen acceptor. ^c Chlorobenzene instead of toluene as a solvent. ^d Reaction performed in a single step: HG 2 and Pd catalysts were placed in a Schlenk tube under an inert atmosphere, solvent and then EPA were added, and the mixture was heated to 110 °C and stirred for 20 h.

The metathesis was not adversely influenced by the presence of a solvent; full and selective conversion to 1,4-cyclohexadiene was retained (ESI⁺). The presence of a residual ruthenium catalyst is not detrimental to the catalytic dehydrogenation, though the conversion to benzene appeared to be slightly affected in the case of using the post-metathesis reaction mixture. This was also found when employing the other previously tested Pd precursors (Table 2, entries 5 and 6).

As the metathesis is the faster reaction, we investigated whether both reactions could be combined into a single step (Table 2, entry 7), that is combining the substrate, solvent and both catalysts in the initial reaction mixture and gradually heating to the dehydrogenation temperature of 110 °C. Unfortunately, the metathesis step was now hampered and did not reach full conversion (38%). The subsequent dehydrogenation did proceed relatively well; all in all a total selectivity towards benzene of 28% based on EPA was achieved.

Although the unsaturated linear C₁₀ diacid and C₈ monoacid are useful compounds, their saturated analogues are often preferred. In particular, sebacic acid is in strong demand. As the dehydrogenation catalyst employed can also affect hydrogenation according to the concept of microscopic reversibility, we probed whether this hydrogenation, however

could be affected in parallel to the formation of benzene by hydrogenation.

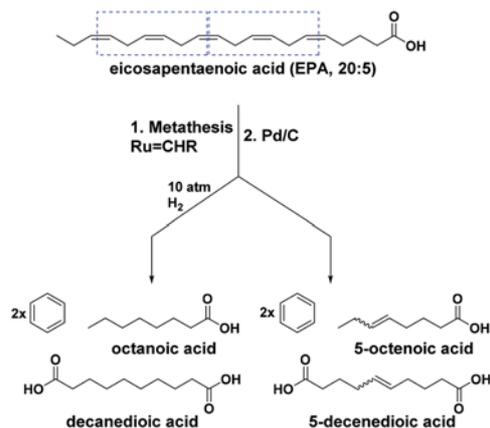
In order to identify suitable reaction conditions, post-metathesis/post-dehydrogenation mixtures were subjected to hydrogenation under H₂ pressure (Table 3, entries 1 and 2). Even at only 5 bar hydrogen pressure, the unsaturated 5-octenoic acid and 5-decenedioic acid were fully hydrogenated. With this insight, the metathesis/dehydrogenation reactions were performed according to the aforementioned protocol, but with hydrogen present during the dehydrogenation step (Table 3, entries 3 to 5).

Indeed, benzene formation still occurs at these hydrogen pressures due to the high driving force of aromatization, while at the same time the linear olefinic compounds are hydrogenated effectively (Scheme 2). Note that the composition of the (saturated) acids is shifted in favour of sebacic acid beyond the 2:1 molar ratio expected from a metathesis equilibrium. Possibly, the diacid is hydrogenated more rapidly, and shifts the ongoing metathesis by this removal of the metathesis product. The hydrogen released in the dehydrogenation to benzene is exactly the amount required to hydrogenate the remaining non-cyclic unsaturated molecules; the partial pressure of the hydrogen evolved apparently is not sufficient to actually perform the olefin hydrogenation.

Table 3 Hydrogenation of mixtures containing benzene formed in the 2-step metathesis and dehydrogenation reactions of 1,4-cyclohexadiene formed in the EPA metathesis reactions

Entry	H ₂ (bar)	Conv. of (found) 1,4-CHD ^a (%)	C ₆ H ₆ select. ^a (%)	Hydrogenation selectivity of linear olefins (%)	Composition of saturated linear acid portion (mol%)		
					8:0 Acid ^a (%)	1,10-Diacid ^a (%)	Other products ^b (%)
Hydrogenation on the post-metathesis, post-dehydrogenation mixture:							
1	5	100	99	100	56	39	5
2	10	100	100	100	44	38	18
Simultaneous post-metathesis dehydrogenation/hydrogenation:							
3	5	100	96	100	25	75	0
4	10	100	100	100	26	67	7
5	1	100	98	89	75	25	0

Conditions: EPA (5 mmol), HG2 (0.005 mmol, 1 mol%), 45 °C, 30 min, then toluene (10 mL), Pd/C (10% w/w, 1 mol%), 110 °C, 20 h. ^a Determined *via* GC analysis. Selectivity was determined with respect to the maximum theoretical amount based on EPA. ^b Other products are the result of incomplete metathesis. These consist mainly of undecadienoates.



Scheme 2 Self-metathesis and the subsequent dehydrogenation of EPA resulting in benzene, 5-decenedioic acid and 5-octenoic acid in the absence of hydrogen. In the presence of even 1 atmosphere hydrogen, benzene and the saturated compound are formed.

Conclusions

The one-pot procedure of catalytic metathesis and dehydrogenation enables an efficient production of benzene from eicosapentaenoic acid. The latter is a component of microalgae oil feedstocks, amongst others. This avenue overcomes the current lack of routes to desirable individual aromatic compounds from renewable sources, rather than complex mixtures of oxygenated aromatics which are also not separable. Benzene is essential for the production of many actives, dyes and other chemicals. As the only major by-products, decenedioic acid and octenoic acid are formed. Remarkably, these can be hydrogenated simultaneously to the dehydrogenation step to form their saturated analogues. In particular, sebacic acid is in strong demand for high-performance polyamides, corrosion protection and other applications.

While the production of benzene from fatty acids is certainly not economically viable today, this scheme does provide a perspective to access this important building block from non-fossil sources efficiently.

Experimental

General considerations

All reactions were performed under an inert gas atmosphere and mixed using a magnetic stirring bar, unless stated otherwise. Chlorobenzene was degassed and stored over molecular sieves under an inert atmosphere, toluene was distilled over Na and stored under an inert atmosphere, and methanol was distilled over Mg and stored over molecular sieves under an inert atmosphere. Ethyl vinyl ether (EVE), 1,4-cyclohexadiene and anthraquinone-2-sulfonic acid sodium salt (AMS) were obtained from Sigma-Aldrich and used as received. Eicosapentaenoic acid (EPA) and the Hoveyda-Grubbs 2nd generation catalyst were purchased from Carbosynth and used

as received. EPA was distilled before use. Pd/C (10%), Pd/Al₂O₃ (5%) and Pd(OAc)₂ were obtained from ABCR. The metathesis catalyst precursors, Grubbs 1st generation, Grubbs 2nd generation, Grubbs 3rd generation and Hoveyda-Grubbs 1st generation, were purchased from Sigma-Aldrich and used as received. CatMETetium was purchased from ABCR. Umicore metathesis catalyst precursors were kindly donated by Umicore. Hydrogen (N5.0, 99.999%) was obtained from Air Liquide. Gas chromatography was performed on a PerkinElmer GC Clarus 500 system equipped with an elite-5 column (length = 30 m, inner diameter = 0.25 mm, thickness = 25 μm) and a FID detector *via* the following programs: 1 min at 90 °C, 30 °C min⁻¹ to 280 °C, 280 °C for 8 minutes (method 1), or 3 min at 50 °C, 20 °C min⁻¹ to 280 °C, 280 °C for 5 minutes (method 2), both with an injector temperature of 300 °C and a detector temperature of 280 °C. GC-MS measurements were conducted on an Agilent GC7890A system equipped with an inert MSD 5975C triple-axis detector. The column in the GC-MS was an HP-5 ms. NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer. ¹H NMR spectra were referenced to the residual protiated solvent (CDCl₃).

Catalysis

Procedure for the metathesis of eicosapentaenoic acid. In the glovebox, the appropriate metathesis catalyst precursor (0.005 mmol, 0.1 mol%) was weighed into a Schlenk tube. The Schlenk tube was closed with a septum and transferred out of the glovebox. Under an inert atmosphere, eicosapentaenoic acid (5 mmol) was added *via* a syringe. At time intervals of 0, 15, 30, 45, 75, 105, 180 and 240 minutes, samples were taken *via* a syringe from the reaction mixture. The sample was quenched with EVE and filtered over silica to remove all metal residues. The sample was subsequently analysed by GC.

Procedure for the dehydrogenation of 1,4-cyclohexadiene. The appropriate Pd source (Pd/C (10% w/w) 5–0.1 mol%, Pd(OAc)₂ 1 mol%, Pd/Al₂O₃ (5% w/w) 1–0.1 mol%) was loaded into a Schlenk tube. In case AMS or dimethylfumarate was applied, these were added (0.2 mmol, 62.1 mg or 1 equiv., 1.44 g, 0.25 equiv., 360 mg, respectively). The Schlenk tube was closed with a septum and evacuated and purged with N₂ 3 times. Using a syringe, the solvent (chlorobenzene or toluene) was added, followed by the addition of 1,4-cyclohexadiene (10 mmol), also *via* a syringe. The reaction mixture was then heated to the stated temperature and stirred for the appropriate time. After this time, the reaction mixture was cooled to room temperature and a sample was taken from the mixture and filtered over silica to remove any catalyst. The sample was analysed by GC.

Procedure for the single-step one-pot metathesis and dehydrogenation

One-step procedure. In a Schlenk tube, Pd/C 10% w/w (0.1 mmol, 1 mol%) was weighed, and the tube was evacuated and purged with an inert gas. To this, Hoveyda-Grubbs 2nd generation catalyst (0.005 mmol, 0.1 mol%) was added in the glovebox. The Schlenk tube was closed with a septum and transferred out of the glovebox. Using a syringe, chlorobenzene

or toluene (10 mL) was added, followed by the addition of eicosapentaenoic acid (5 mmol). The mixture was heated to 110 °C over the course of 25 minutes and then stirred at this temperature for 20 h. After the reaction mixture was cooled to room temperature, a sample was filtered over silica and analysed by GC.

Procedure for the two-step one-pot metathesis and dehydrogenation. In a Schlenk tube, the Hoveyda–Grubbs 2nd generation catalyst (0.005 mmol, 0.1 mol%) was weighed in the glovebox. The Schlenk tube was closed with a septum and transferred out of the glovebox. Using a syringe, chlorobenzene or toluene (10 mL) was added. The mixture was heated to 45 °C and eicosapentaenoic acid (5 mmol) was added. The mixture was stirred for 15 min. After this, Pd/C 10% w/w (0.1 mmol, 1 mol%) was added and the entire mixture was further heated to 110 °C and stirred at this temperature for 20 h. After the reaction mixture was cooled to room temperature, a sample was filtered over silica and analysed by GC.

Procedure for the two-step one-pot metathesis and dehydrogenation in the presence of H₂. In a Schlenk tube, the Hoveyda–Grubbs 2nd generation catalyst (0.005 mmol, 0.1 mol%) was weighed in the glovebox. The Schlenk tube was closed with a septum and transferred out of the glovebox. Using a syringe, preheated eicosapentaenoic acid (5 mmol) was added. The mixture was stirred for 15 min. A balloon filled with H₂ was connected to the Schlenk tube *via* a needle. A suspension of Pd/C 10% w/w (0.1 mmol, 1 mol%) in toluene (10 mL) was added and the entire mixture was further heated to 110 °C and stirred at this temperature for 20 h. After the reaction mixture was cooled to room temperature, a sample was filtered over silica and analysed by GC.

In the case of the experiments with 5 and 10 bar H₂, the metathesis mixture was transferred to a stainless steel autoclave and a suspension of Pd/C 10% w/w (0.1 mmol, 1 mol%) in toluene (10 mL) was added. The autoclave was pressurized with the appropriate H₂ pressure and heated to 110 °C for 20 h. After the reaction mixture was cooled to room temperature, a sample was filtered over silica and analysed by GC.

Conflicts of interest

The authors declare no conflict of interests.

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Notes and references

1 A. Kaiho, M. Kogo, R. Sakai, K. Saito and T. Watanabe, *Green Chem.*, 2015, **17**, 2780.

- 2 C. A. Gasser, M. Čvančarová, E. M. Ammann, A. Schäffer, P. Shahgaldian and P. F.-X. Corvini, *Appl. Microbiol. Biotechnol.*, 2017, **101**, 2575.
- 3 J. Zakzeski, P. C. A. Bruijninx, A. L. Jongerius and B. M. Weckhuysen, *Chem. Rev.*, 2010, **110**, 3552.
- 4 R. J. Winwood, *OCL*, 2013, **20**, D604.
- 5 M. L. Hamilton, R. P. Haslam, J. A. Napier and O. Sayanova, *Metab. Eng.*, 2014, **22**, 3.
- 6 Z. Xue, P. L. Sharpe, S.-P. Hong, N. S. Yadav, D. Xie, D. R. Short, H. G. Damude, R. A. Rupert, J. E. Seip, J. Wang, D. W. Pollak, M. W. Bostick, M. D. Bosak, D. J. Macool, D. H. Hollerbach, H. Zhang, D. M. Arcilla, S. A. Bledsoe, K. Croker, E. F. McCord, B. D. Tyreus, E. N. Jackson and Q. Zhu, *Nat. Biotechnol.*, 2013, **31**, 734.
- 7 P. Mäki-Arvela, I. Kubickova, M. Snåre, K. Eränen and D. Y. Murzin, *Energy Fuels*, 2007, **21**, 30.
- 8 C. Huang, X.-F. Chen, L. Xiong, X.-D. Chen, L.-L. Ma and Y. Chen, *Biotechnol. Adv.*, 2013, **31**, 129.
- 9 P. Roesle, F. Stempfle, S. K. Hess, J. Zimmerer, C. Río Bártulos, B. Lepetit, A. Eckert, P. G. Kroth and S. Mecking, *Angew. Chem., Int. Ed.*, 2014, **53**, 6800.
- 10 Y. Chen, N. Zhao, Y. Wu, K. Wu, X. Wu, J. Liu and M. Yang, *Environ. Prog. Sustainable Energy*, 2017, **36**, 259.
- 11 T. M. Mata, A. A. Martins and N. S. Caetano, *Renewable Sustainable Energy Rev.*, 2010, **14**, 217.
- 12 C. Ratledge, *OCL*, 2013, **20**, D602.
- 13 S. K. Hess, B. Lepetit, P. G. Kroth and S. Mecking, *Eur. J. Lipid Sci. Technol.*, 2018, **120**, 1700152.
- 14 R. T. Mathers, *Plant based monomers and polymers*, US20130324688A1, 2013.
- 15 P. J. Deuss, K. Barta and J. G. de Vries, *Catal. Sci. Technol.*, 2014, **4**, 1174.
- 16 S. Chikkali and S. Mecking, *Angew. Chem., Int. Ed.*, 2012, **51**, 5802.
- 17 J. A. M. Lummiss, K. C. Oliveira, A. M. T. Pranckevicius, A. G. Santos, E. N. dos Santos and D. E. Fogg, *J. Am. Chem. Soc.*, 2012, **134**, 18889.
- 18 C. S. Higman, M. P. de Araujo and D. E. Fogg, *Catal. Sci. Technol.*, 2016, **6**, 2077.
- 19 R. J. Rico, M. Page and C. Doubleday, *J. Am. Chem. Soc.*, 1992, **114**, 1131.
- 20 G. Fochi, *Organometallics*, 1988, **7**, 2255.
- 21 R. T. Mathers, M. J. Shreve, E. Meyler, K. Damodaran, D. F. Iwig and D. J. Kelley, *Macromol. Rapid Commun.*, 2011, **32**, 1338.
- 22 E. Verkuijlen and C. Boelhouwer, *J. Chem. Soc., Chem. Commun.*, 1974, 793.
- 23 J. Lever, K. Wagner and J. Sworen, *Simplified methods of making 1,3-cyclohexadiene*, US20030176749A1, 2003.
- 24 D. Q. Hu, C. D. MacPherson and K. T. Leung, *Surf. Sci.*, 1992, **273**, 21.
- 25 A. V. Iosub and S. S. Stahl, *ACS Catal.*, 2016, 8201.