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COMMUNICATION

Development of Site- and Stereoselective Continuous Flow Deuterium Labelling Method for Carbohydrates Using High Dispersion Effect towards Ru/C of Hydrogen Flow

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A site- and stereoselective deuterium labelling method for carbohydrates has been developed using a Ru/C catalyst under continuous flow conditions. It has been demonstrated that enhancing the void fraction of the catalyst cartridge leads to improved incorporation, while maintaining high selectivity over 150 h. This scalable, sustainable approach has the potential to reduce energy use, waste and Ru consumption, thus broadening continuous-flow applications in organic synthesis.

Deuterium-labelled compounds find application in a variety of fields, including NMR spectroscopy, pharmacokinetic studies, and environmental analysis.[1–5] Carbohydrates, in particular, are indispensable building blocks in the synthesis of bioactive molecules.[6] Deuterated carbohydrates serve as key materials for deuterium metabolic imaging, a non-invasive method combining non-radioactive deuterium-labelled substrates with deuterium magnetic resonance imaging.[7–11] This approach can create three-dimensional metabolic maps,[7] aid in distinguishing tumour tissues,[9–10] and investigate hepatic fructose and glucose metabolism.[8] Scalable synthesis methodologies for deuterated carbohydrates are therefore essential in these fields.

Enzymatic synthesis methods[12] and ketone reduction in oxidised carbohydrates using $LiAlD_4$ or $NaBD_4$[13,14] have been effective for preparing deuterated carbohydrates, but they are costly, time-consuming, and generate by-products. Direct deuteration techniques employing Raney Ni as a catalyst in D_2O under a hydrogen atmosphere have limited applicability and yield relatively low deuterium incorporation efficiencies.[15,16] Furthermore, stringent and extended refluxing conditions have been shown to readily facilitate hydrolysis, racemisation, and epimerisation.[13] Alternatively, we have developed a practical method for deuterium labelling of organic molecules under a hydrogen atmosphere in D_2O using heterogeneous platinum-group catalysts.[2,17–20] It is important to note that this method involves a site- and stereoselective deuterium labelling strategy for carbohydrates. This strategy employs Ru/C as the catalyst, which has recently attracted attention due to its high activity and ongoing development efforts (Figure 1a).[17,19,21,22]

Continuous flow reactions using catalyst-packed cartridges enhance the efficiency, safety, operational simplicity, and scalability of organic synthesis. Such methods have been shown to enhance interaction with catalysts, thereby facilitating large-scale synthesis by extending reaction duration.[23–25] Significant advances have been made in methodologies employing continuous flow systems with packed heterogeneous catalysts, including flow-based H–D exchange strategies for deuterium labelling and site-selective synthesis of deuterium-labelled β-nitroalcohols.[20,26–30] Furthermore, related catalytic work performed under continuous flow conditions emphasises the innovation and extensive applicability of the proposed approach, highlighting how meticulously optimised catalyst systems can substantially enhance overall performance.[31] Unlike batch methods, the deuterium-labelled product generated by the reaction is immediately flushed out of the catalyst cartridge in the continuous flow method. By limiting the contact time between the product and catalyst, undesired side reactions can be prevented.

In this method, a minute quantity of Ru/C is charged in proportion to the volume of the 3-mm diameter catalyst cartridge, thereby creating a substantial void (space) within the cartridge that is not occupied by Ru/C. Hydrogen gas is introduced into the cartridge in an upward direction, along with a D_2O solution of carbohydrates. This allows the D_2O solution to fill much of the unoccupied space within the catalyst cartridge. The higher flow rate of hydrogen compared to the reaction solution facilitates agitation of the Ru/C layer in the accumulated flow reaction solution within the cartridge, resulting in uniform dispersion of Ru/C. The D_2O solution replaces a conventional solid diluent typically employed in flow reactions, thereby forming a catalytic reaction zone in which Ru/C is almost uniformly dispersed. This facilitates efficient interaction between the

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substrate and the Ru–D_2O interface. This enables site- and stereoselective H–D exchange at the carbon atoms adjacent to hydroxyl groups (Figure 1b). Increasing the cartridge void fraction improves the deuterium labelling rate whilst maintaining both site- and stereoselectivity. This approach achieves >90% deuterium incorporation, maintains stable catalytic performance over 150 h and minimises resource consumption. This highlights the importance of catalyst dispersion in optimising H–D exchange efficiency under continuous flow conditions.

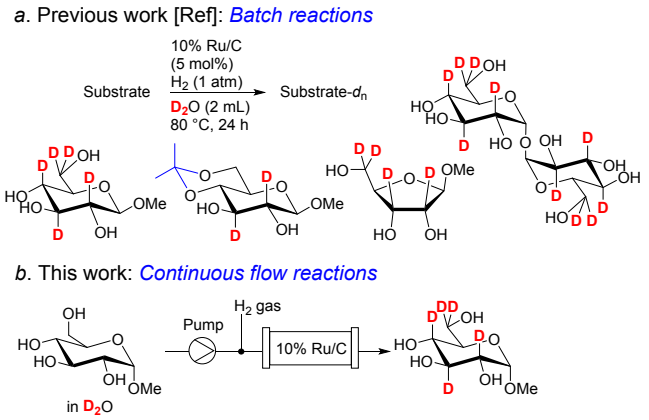

Figure 1. Ru/C-catalysed deuterium labelling method for carbohydrates. (a) Batch reaction. (b) Continuous flow reaction.

The investigation commenced by evaluating the impact of the catalyst cartridge temperature and the quantity of 10% Ru/C on the deuterium labelling efficiency of methyl-α-D-glucopyranoside (**1a**) (Figure 2). Initially, 10% Ru/C (130 mg) mixed with Celite (300 mg) as a diluent was packed into a cartridge (Φ: 3.0 mm, L: 200 mm) to examine the deuteration of **1a** under various continuous flow conditions. However, this configuration resulted in flow interruptions caused by cartridge clogging. To address this, a solution of **1a** in D_2O (0.125 M, flow rate: 0.05 mL min^{-1}) and hydrogen gas (flow rate: 0.8 mL min^{-1}) were introduced in up-flow into a cartridge containing only 10% Ru/C (130 mg) at 80 °C for 4 h. Under optimised conditions, deuterium atoms were selectively incorporated at the C2, C3, C4, and C6 positions adjacent to the hydroxyl groups, yielding multi-deuterated methylglycoside **1a**[D_5] without clogging. The product was isolated as its tetraacetate derivative **2a**. The chirality of **2a** was preserved under these reaction conditions, as confirmed by ^{1}H NMR analysis, and its deuterium content ranged from 51% to 92% after 4 h (Figure 2a). The deuterium incorporation efficiency improved as the reaction temperature was raised from 80 to 100 °C, reaching 72–95% D after 4 h (Figure 2b). Similarly, increasing the amount of 10% Ru/C catalyst from 130 mg to 250 mg further enhanced the labelling efficiency to 90–97% after 4 h (Figure 2c). Conversely, shortening the catalyst cartridge from 200 mm to 50 mm or increasing the substrate solution concentration to 0.25 M diminished the deuterium content of **2a**.

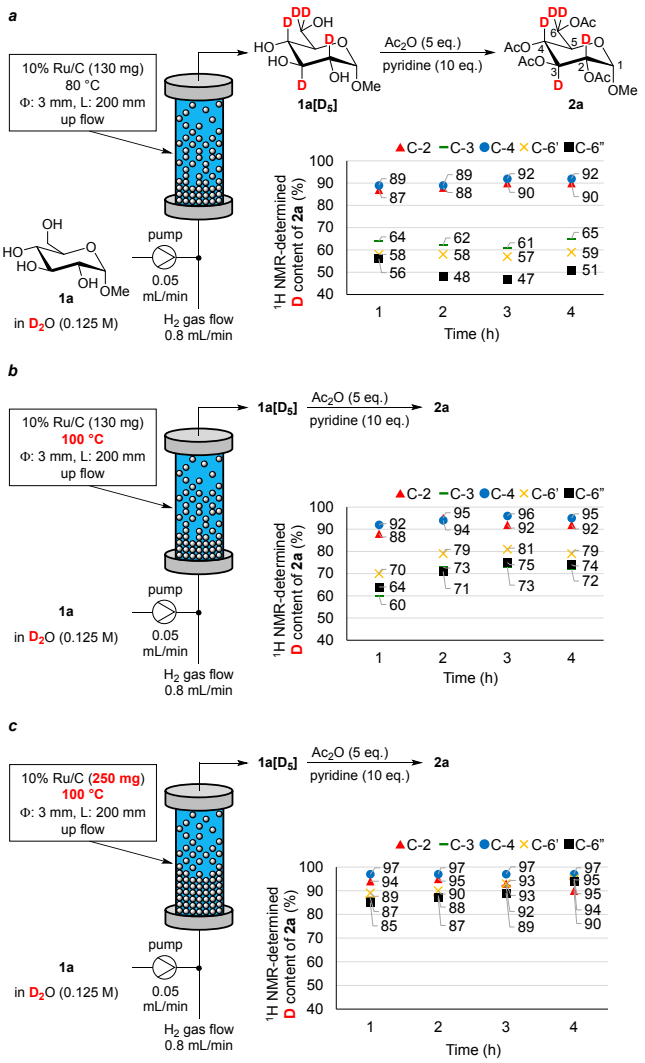

Figure 2. Optimisation of conditions for continuous flow deuteration of methyl-α-D-glucopyranoside. (a) Conducted at 80 °C using 130 mg of 10% Ru/C. (b) Conducted at 100 °C using 130 mg of 10% Ru/C. (c) Conducted at 100 °C using 250 mg of 10% Ru/C.

Subsequently, we investigated the deuterium labelling efficiency using a new substrate, methyl-α-D-mannopyranoside (**1b**) (Figure 3). A solution of **1b** in D_2O (0.125 M, flow rate: 0.05 mL min^{-1}) and hydrogen gas (flow rate: 0.8 mL min^{-1}) were introduced into a cartridge (Φ: 3.0 mm, L: 200 mm) containing 10% Ru/C (250 mg) at 100 °C for 4 h. Under these conditions, the deuterium labelling efficiency was moderate, with a D content of 73–79% (Figure 3a). Increasing the catalyst amount from 250 mg to 400 mg did not enhance labelling efficiency, as the D content remained at 69–73% (Figure 3b). By contrast, a marked improvement was observed when two catalyst cartridges (Φ: 3.0 mm, L: 200 mm each), connected in series, were each packed with 10% Ru/C (125 mg). Under these conditions, the D content remained at 90–97% for 4 h (Figure 3c). These findings suggest that increasing the void fraction within the catalyst cartridge, alongside the high dispersion conditions of 10% Ru/C, promotes efficient mixing of

the substrate solution, H_2 gas, and 10% Ru/C, thereby enabling a highly efficient H–D exchange reaction.

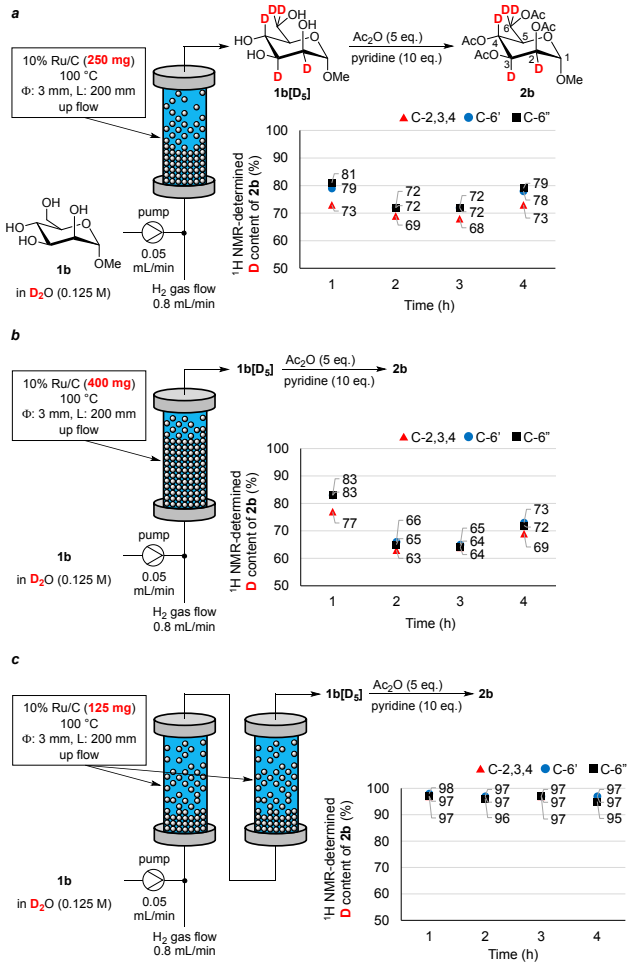

Figure 3. Optimisation of conditions for continuous flow deuteration of methyl-*α*-D-mannopyranoside using (a) a cartridge containing 250 mg of 10% Ru/C, (b) a cartridge containing 400 mg of 10% Ru/C, and (c) two cartridges, each containing 125 mg of 10% Ru/C.

Further investigations explored the deuteration of additional carbohydrates (Figure 4). Products formed during the first hour of the continuous flow reaction were discarded, and yields, as well as deuteration efficiency, were determined from the products collected in each position over the subsequent 3 h. During the initial hour of the experiment, it was observed that the catalyst cartridge had not been adequately saturated with a mixture of H_2 and the D_2O–substrate solution. Consequently, the effective substrate concentration within the cartridge did not attain the designated level of 0.125 M. These observations were identified as the fundamental cause of the decline in yield and deuteration efficiency. Notably, the D content of **2a**, derived from **1a**, remained stable between 92% and 95% for three hours. The partial decomposition of methyl-*α*-L-fucopyranoside (**1c**) and methyl-*β*-D-ribofuranoside (**1d**) was mitigated by the addition of LiOH (see ESI‡). A solution of the substrate and LiOH (2 equiv) in D_2O (0.125 M, flow rate: 0.05 mL min^{-1}) and H_2 gas (flow rate: 0.8 mL min^{-1}) was passed through a cartridge (Φ: 3.0 mm, L: 200 mm) containing 10% Ru/C (250 mg) at 100 °C for 4 h. Under the optimised conditions, **2c** exceeded 95% D content for three hours, whilst **2d** reached 70–95% over 5 h using two 5.0 mm catalyst cartridges. These findings suggest that this continuous flow method effectively introduces deuterium into carbohydrates, which are crucial for pharmaceuticals and bioactive compounds.

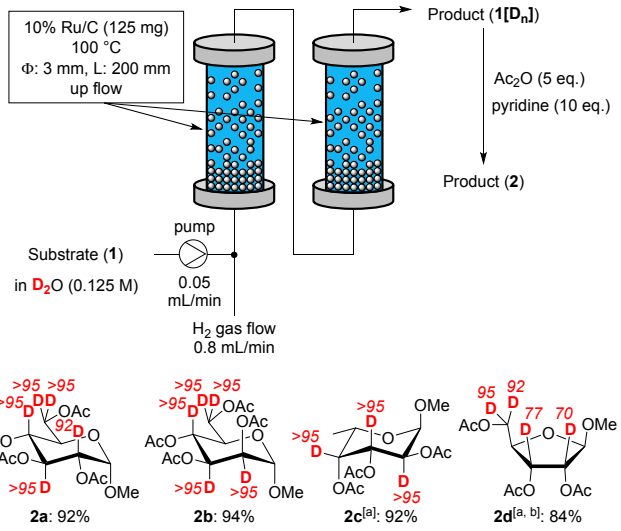

Figure 4. Ru/C-catalysed site- and stereoselective H–D exchange reaction of various carbohydrates. Italicised numbers indicate the deuterium efficiencies. [a] 2 equiv of LiOH was used. [b] The apparatus consisted of two catalyst cartridges, each with an inner diameter of 5.0 mm and a length of 200 mm, connected in series.

To assess the performance of the system over prolonged continuous flow conditions, a D_2O solution of **1a** (0.125 M, flow rate: 0.05 mL min^{-1}) and H_2 gas (flow rate: 0.8 mL min^{-1}) were continuously passed through two cartridges (Φ: 3.0 mm, L: 200 mm) packed with 125 mg of 10% Ru/C at 100 °C, connected in series (Figure 5). The deuterium labelling efficiency was monitored at 24-hour intervals. The reaction proceeded continuously for at least 150 h without any discernible loss in catalytic activity. The deuterium incorporation rate for **2a** remained consistently between 91% and 95%, yielding 19.5 g (95%) of isolated **2a**. Moreover, a turnover number (TON) of 1021, a turnover frequency (TOF) of 6.8 h^{-1}, and a space-time yield (STY) of 4.5 mol $Lcat^{-1}$ h^{-1} were achieved, demonstrating the system's robustness and efficiency for continuous applications. Analysis of the eluate by inductively coupled plasma atomic emission spectrometry (ICP-AES, detection limit: 1.5 ppb) confirmed that no ruthenium species were present.

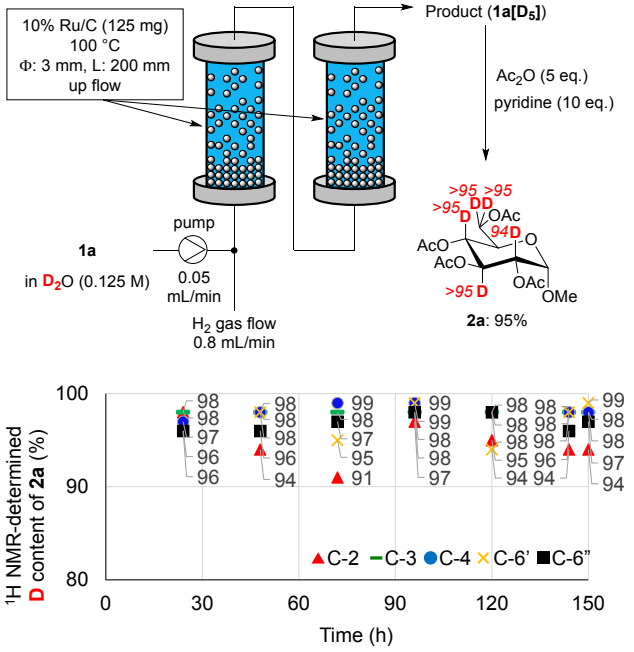

Figure 5. Long-term continuous flow deuterium labelling of **1a** using a 10% Ru/C-packed catalyst cartridge.

Conclusions

In conclusion, a highly efficient continuous flow method for the site- and stereoselective deuteration of carbohydrates was developed using a Ru/C catalyst under mild conditions. The deuterium labelling efficiency was markedly improved by increasing the void fraction within the catalyst cartridge, whilst maintaining high selectivity. This underscores the vital role of effective mixing and extended reagent contact time. Notably, the catalytic activity was sustained over 150 h of continuous deuteration of **1a**, achieving >90% deuterium incorporation for **2a** and a TON of 1021. Compared with conventional batch processes, this method offers significant benefits, including enhanced sustainability by reducing energy, chemicals, labour, and material waste, whilst conserving Ru, a precious metal resource. It provides a scalable and cost-effective approach for synthesising deuterated carbohydrates, valuable for diverse applications. The findings highlight the potential of continuous-flow methodologies in organic synthesis to improve the efficiency, scalability, and sustainability of isotope labelling processes.

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Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the ESI.

Notes and references

1 J. Atzrodt, V. Derdau, W. J. Kerr, M. Reid, *Angew. Chem. Int. Ed.*, 2018, **57**, 1758–1784.
2 Y. Sawama, K. Park, T. Yamada, H. Sajiki, *Chem. Pharm. Bull.*, 2018, **66**, 21–28.
3 S. Kopf, F. Bourriquen, W. Li, H. Neumann, K. Junge, M. Beller, *Chem. Rev.*, 2022, **122**, 6634–6718.
4 R. D. Jansen-van Vuuren, L. Jedlovčnik, J. Košmrlj, T. E. Massey, V. Derdau, *ACS Omega*, 2022, **7**, 41840–41858.
5 H. Li, M. Shabbir, W. Li, A. Lei, *Chin. J. Chem.*, 2024, **42**, 1145–1156.
6 H. Gabius, H. Siebert, S. André, J. Jiménez‐Barbero, H. Rüdiger, *ChemBioChem*, 2004, **5**, 740–764.
7 H. M. De Feyter, K. L. Behar, Z. A. Corbin, R. K. Fulbright, P. B. Brown, S. McIntyre, T. W. Nixon, D. L. Rothman, R. A. De Graaf, *Sci. Adv.*, 2018, **4**, eaat7314.
8 A. D. Hendriks, A. Veltien, I. J. Voogt, A. Heerschap, T. W. J. Scheenen, J. J. Prompers, *Front. Physiol.*, 2023, **14**, 1198578.
9 E. T. Montrazi, K. Sasson, L. Agemy, D. C. Peters, O. Brenner, A. Scherz, L. Frydman, *Sci. Rep.*, 2023, **13**, 19998.
10 K. M. Brindle, *Npj Imaging*, 2024, **2**, 1.
11 E. T. Montrazi, K. Sasson, L. Agemy, A. Scherz, L. Frydman, *Sci. Adv.*, 2024, **10**, eadm8600.
12 P. Vallurupalli, L. Scott, M. Hennig, J. R. Williamson, L. E. Kay, *J. Am. Chem. Soc.*, 2006, **128**, 9346–9347.
13 J. J. De Voss, J. J. Hangeland, C. A. Townsend, *J. Org. Chem.*, 1994, **59**, 2715–2723.
14 M. K. Kundu, A. Földesi, J. Chattopadhyaya, *Helv. Chim. Acta*, 2003, **86**, 633–643.
15 E. A. Cioffi, R. H. Bell, B. Le, *Tetrahedron Asymmetry*, 2005, **16**, 471–475.
16 J. R. Heys, *J. Label. Compd. Radiopharm.*, 2010, **53**, 716–721.
17 Y. Fujiwara, H. Iwata, Y. Sawama, Y. Monguchi, H. Sajiki, *Chem. Commun.*, 2010, **46**, 4977–4979.
18 Y. Sawama, Y. Monguchi, H. Sajiki, *Synlett*, 2012, **23**, 959–972.
19 Y. Sawama, Y. Yabe, H. Iwata, Y. Fujiwara, Y. Monguchi, H. Sajiki, *Chem. Eur. J.*, 2012, **18**, 16436–16442.
20 K. Park, N. Ito, T. Yamada, H. Sajiki, *Bull. Chem. Soc. Jpn.*, 2021, **94**, 600–605.
21 X. Yang, H. Ben, A. J. Ragauskas, *Asian J. Org. Chem.*, 2021, **10**, 2473–2485.
22 X. Wang, X. Yang, J. Sun, M. Guo, Z. Cao, H. Ben, W. Jiang, S. Ming, L. Zhang, *Chem. Commun.*, 2023, **59**, 6544–6547.
23 T. Tsubogo, H. Oyamada, S. Kobayashi, *Nature*, 2015, **520**, 329–332.
24 J. Britton, C. L. Raston, *Chem. Soc. Rev.*, 2017, **46**, 1250–1271.

25 M. B. Plutschack, B. Pieber, K. Gilmore, P. H. Seeberger, *Chem. Rev.*, 2017, **117**, 11796–11893.
26 T. Hattori, A. Tsubone, Y. Sawama, Y. Monguchi, H. Sajiki, *Catalysts*, 2015, **5**, 18–25.
27 T. Hattori, T. Ida, A. Tsubone, Y. Sawama, Y. Monguchi, H. Sajiki, *Eur. J. Org. Chem*., 2015, **11**, 2492–2497.
28 T. Yamada, K. Park, N. Ito, H. Masuda, W. Teranishi, S. Cui, H. Sajiki, *Bull. Chem. Soc. Jpn.*, 2020, **93**, 1000–1006.
29 T. Yamada, K. Park, C. Furugen, J. Jiang, E. Shimizu, N. Ito, H. Sajiki, *ChemSusChem*, 2022, **15**, e202102138.
30 N. Sakurada, K. Kobayashi, Y. Abe, K. Niwa, T. Yokoyama, T. Yamada, T. Ikawa, H. Sajiki, *ChemSusChem*, 2024, **18**, e202401859.
31 X. Yang, J. Yang, T. Zhao, W. Qian, Y. Wang, A. Holmen, W. Jiang, D. Chen, H. Ben, *Chem. Eng. J.*, 2022, **445**, 136655.

A statement of data availability has been included at the end of the article under the heading "Data availability", after the conflict of interest statement and before the acknowledgements, as shown below.

The data supporting this article have been included as part of the ESI.