Concise asymmetric synthesis of (−)-bilobalide

The leaves of *Ginkgo biloba* have been used historically as insecticides and helminthicides, and this activity has been attributed to their constituent terpene trilactones, including bilobalide (1, Fig. 1). *Ginkgo* extracts have also been used in traditional Chinese medicine to treat senility, a practice that has penetrated the Western world—although not without controversy, owing to opposing claims of efficacy and serious adverse effects associated with Ginkgo toxin (4-O-methylpyridoxine) or the inhibition of platelet aggregating factor by ginkgolides. Animal models demonstrate some credible effects on impaired cognition: in a mouse model of Down syndrome (Ts65Dn), in which mice show deficits in declarative learning and memory, normalized novel object recognition was exhibited after treatment with pure bilobalide. Rescue of learning and memory is proposed to arise through neuronal excitation by antagonism of GABA\(_\text{ARs}\.\) Unlike the plant metabolite picrotoxinin (2, Fig. 1), bilobalide is not acutely toxic, and unlike the ginkgolides, bilobalide does not affect platelet aggregating factor. Despite their disparate toxicity, bilobalide and picrotoxinin exhibit similar inhibitory potencies at recombinant GABA\(_\text{ARs}\), with half-maximum inhibitory concentrations (IC\(_{50}\)) of 4.6 μM and 2.0 μM, respectively (α1β2γ2L receptor). Bilobalide1,2,6 has prevented pull-down of biological targets other than the GABA\(_\text{ARs}\). A concise and flexible synthesis of bilobalide would facilitate the development of probes for the identification of potential new targets, analogues with differential selectivity between insect and human GABA\(_\text{ARs}\), and stabilized analogues with an enhanced serum half-life. Here we exploit the unusual reactivity of bilobalide to enable a late-stage deep oxidation that symmetrizes the molecular core and enables oxidation states to be embedded in the starting materials. The same overall strategy may be applicable to *G. biloba* congeners, including the ginkgolides—some of which are glycine-receptor-selective antagonists. A chemical synthesis of bilobalide should facilitate the investigation of its biological effects and its therapeutic potential.

The *Ginkgo biloba* metabolite bilobalide is widely ingested by humans but its effect on the mammalian central nervous system is not fully understood. Antagonism of \(\gamma\)-aminobutyric acid A receptors (GABA\(_\text{ARs}\)) by bilobalide has been linked to the rescue of cognitive deficits in mouse models of Down syndrome. A lack of convulsant activity coupled with neuroprotective effects has led some to postulate an alternative, unidentified target; however, steric congestion and the instability of bilobalide have prevented pull-down of biological targets other than the GABA\(_\text{ARs}\). A concise and flexible synthesis of bilobalide would facilitate the development of probes for the identification of potential new targets, analogues with differential selectivity between insect and human GABA\(_\text{ARs}\), and stabilized analogues with an enhanced serum half-life. Here we exploit the unusual reactivity of bilobalide to enable a late-stage deep oxidation that symmetrizes the molecular core and enables oxidation states to be embedded in the starting materials. The same overall strategy may be applicable to *G. biloba* congeners, including the ginkgolides—some of which are glycine-receptor-selective antagonists. A chemical synthesis of bilobalide should facilitate the investigation of its biological effects and its therapeutic potential.

The synthesis commenced with a methodological challenge: an asymmetric Reformatsky reaction between 6a and 6b (which are produced in two steps and one step respectively, see Supplementary Information section 3). Reformatsky conditions proved necessary owing to the tendency of 7 to undergo retro-aldol cleavage under basic conditions, whereas zinc, chromium and samarium alkoxides were stable at −78°C. To our knowledge, there have been no examples of catalytic enantio- and diastereoselective zinc Reformatsky reactions, nor the use of simple, chiral \(\ell\)-type bisoxazoline (BOX) ligands. A previous study demonstrated the use of related, electron-rich hemiaminals for the control of single stereocentres, and the simplicity of these conditions provided a foundation to explore. After a ligand screen, we found that a combination of diethylzinc and indabox (10 mol% A) provided secondary alcohol 7 in 97:3 enantiomeric ratio (e.e.) in favour of syn-diastereomer 7 (2:3:1). The combined yield of both diastereomers was determined to be 64% by NMR, and the crude reaction mixture could be carried forward efficiently (purification by chromatography...
on silica led to a loss of material via a retro-aldol reaction. The doubly activated bromide 6a may uniquely enable the use of simple BOX ligands. A Giese-type 5-exo-trig cyclization of 7 occurred with high regio- and diastereoselectivity (20:1 diastereoselective ratio (d.r.); the 6-endo-trig product was not detected) to form the quaternary carbon of cyclopentene 8. The material was purified by recrystallization to >99% e.e. and a 21% yield was achieved over two steps; the relative and absolute stereochemistry of the product were confirmed by X-ray crystallography (see Supplementary Information section 5).

Previous syntheses of 1 installed the extremely hindered tert-alkyl, bis-neo-pentyl C8 hydroxyl either by late-stage dihydroxylation with stoichiometric osmium tetroxide (23 °C, 12 h); or by early-stage nitrile anion addition to a tert-butyl ketone, which rendered the syn-stereoselective product (Fig. 2b, entry 4). We recently discovered that the kinetically relevant reductant in many Mukaiyama reactions is an alkoxysilane (for example, Phi-(PrO)SiH) that is formed in situ by silane alcoholysis. This custom, commercially available silane enabled us to screen a diverse range of solvents, and we identified a correlation between solvent polarity and diastereoselectivity, possibly due to internal hydrogen bonding (Fig. 2b). Variation of the ligands on the metal catalyst had no effect on the diastereomeric ratio of the product. The use of tert-butyl methyl ether favoured the wrong (S)-C8 diastereomer of the alcohol (which cyclized to a lactone), whereas methylcyclohexene reversed this stereoselectivity to favour the (R)-C8 diastereomer 9 in a 3:1 ratio.

Formation of the fourth contiguous, fully substituted carbon atom by alkylation was frustrated by the dehydration of 9 under basic conditions or by a preference for the wrong C5 stereoisomer. However, treatment of 9 with strong acid, such as p-toluenesulfonic acid (PTSA) (Fig. 2c) led to unexpectedly stable oxetane acetals 10 (endo-OMe) and 11 (exo-OMe). Stabilization of 10 and 11 is possibly driven by steric compression of the alcohol and acetal carbons—a “corset effect” that increases the energy barrier to ring-opening of strained molecules such as tetrahedranes. Only the minor endo-isomer 10 could be carried forward: the major exo-OMe diastereomer 11 dehydrated under basic conditions and its epimerization to 10 was unsuccessful. However, early racemic route scouting had provided a possible way forward. Screening a library of scalmic binolphosphoric acids had been expected to improve diastereoselectivity, but this effort yielded unsatisfactory results: a 1:7:1 preference for 10 using catalyst B. Analysis by chiral chromatography indicated that a moderate parallel kinetic resolution of (rac)-9 had occurred: each diastereomer possessed opposite enantiomeric excess (10, 39:61 e.r. compared with 11, 69:31 e.r.). Accordingly, a single enantiomer of 9, if matched with the correct enantiomer of chiral acid, would favour formation of the desired endo-10. Indeed, whereas (−)-9 reacted with (−)-B to favour (1.4:1) exo-acetal 11, (−)-9 reacted to favour (4.5:1) endo-acetal 10. Isolated in 71% yield as a single enantiomer, endo-Acetal 10 proved crucial to control the formation of the final quaternary carbon.

Owing to extreme steric hindrance in substrate 10, the final C–C bond could be established only using an alkyne electrophile. A threestep sequence was run in quick succession owing to intermediate instability; only alkyne 12 could be purified. First, oxidation using 2-iodoxygenbenzoic acid (IBX) delivered an unstable β-keto ester that could undergo a-alkylation. Second, this mixture of keto-enol tautomers was treated with Waser’s reagent (1-(trimethylsilyl)ethyl)-1,2-benziodoxol-3(1H)-one; TMS-EBX) and tetra-n-butlammonium fluoride (TBAF). The endo-methoxy oxetane effectively shielded one trajectory of electrophile approach and provided the product as a single diastereomer. By contrast, the exo-methoxy oxetane—if carried forward to this step—eliminated the tert-alkyl ether and did not undergo alkylation. Alkylation before oxetane formation delivered exclusively the incorrect diastereomer. The unstable alkynylation product was reduced with high stereoselectivity using samarium iodide (SmI₂) in a mixture of tetrahydrofuran/water to provide the stable alcohol 12 in 60% yield over three steps. Traditional hydride reductants produced the opposite diastereomer. Anti-Markovnikov hydration of the alkyne to directly incorporate the northern lactone motif could not be accomplished under various standard conditions, including oxidation by lithium tert-butyloxide, so an alternative procedure was developed. Deprotonation of the terminal alkynyl with lithium bis(trimethylsilyl)amide (LiHMDS) followed by treatment with trimethylborate led to the formation of an alkynylborate intermediate. Addition of meta-chloroperbenzoic acid (m-CPBA) to the reaction mixture probably formed an intermediate ketene and/or mixed anhydride that was captured by the adjacent alcohol. To the best of our knowledge, this procedure for alkyn oxidation has not yet been reported. Hydrogenolysis of the benzyl esters followed by in situ acidic hydrolysis caused skeletal rearrangement to (−)-5 in excellent yield.

Introduction of the final, deep C10 oxygen proved challenging. The steric hindrance and ‘bowl’ shape of 5, in addition to its base-lability, detailed many potential solutions. Compound 5 contains three acidic sites—the hydroxyl, the inner lactone and the outer lactone—but the addition of 3 equivalents of strong base, followed by an acetic quench at ~78 °C, caused considerable decomposition and poor mass recovery. We found that treatment with one equivalent of potassium bis(trimethylsilyl)amide (KHMDMS) followed by the addition of aqueous 1M HCl enabled full mass recovery; however, it resulted in the clean delivery of the iso-bilobalide scaffold (for example, 16a, Fig. 3) as a result of intramolecular transesterification. Similarly, we observed transmethylation at room temperature when using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (bilobalide to iso-bilobalide, see Supplementary Information section 3). This facile rearrangement is probably driven.
both by the proximity of the C8 hydroxyl to C4 at the Bürgi–Dunitz angle and by the delocalization of the lactone π system into the adjacent C–O σ* orbital. Molecular models revealed that the iso-bilobalide rearrangement partially folds the skeletal cavity ‘inside out’ (see Fig. 3) to render the inner lactone more accessible to reagents. However, neither the alkoxide of 1 nor its tert-butylmethylsilyl (TBS) ether (16b) provided substantial bilobalide upon treatment with one equivalent each of base and oxidant (Davis’ oxaziridine, (+)-17). Despite the unfolding of the bilobalide cavity and exposure of the C10-proton (highlighted in green), treatment with base still favoured deprotonation of the outer lactone and therefore oxidation provided the isomeric neo-bilobalide 15. We wondered if inner lactone deprotonation required both increased exposure (rearrangement) and increased acidification. Induction through α bonds or delocalization of the lactone π system into an adjacent, withdrawn C–O σ* orbital might acidify the α-protons—that is, stabilize the corresponding enolate30. Conversion of 5 to the isomeric benzoate followed by deprotonation and oxidation at low temperature yielded (−)-1 with only a trace of (+)-1. The same overall strategy disclosed here could not be detected.

The sequence in Fig. 2 has been completed in seven days by one person to produce 0.35 g of (−)-5. The same overall strategy disclosed here may be applicable to G. biloba congeners including the ginkgolides, some of which are glycine-receptor-selective antagonists3. These
Fig. 3 | Late-stage, regio- and stereoselective oxidation of C10 over C1.
Reagents and conditions for entry 3: (1) EDCI, BzOH, DMAP
THF, –78 °C 2 M HCl, 60 °C
1. KHMDS, (±)-[^1–*^]
THF, –78 °C
2 M HCl, 60 °C
6% 94%
Entry 2
1. TBSOTf, 2,6-lut
(KHMDS, (±)-[^1–*^]
THF, –78 °C
2 M HCl, 60 °C
91% 9%
Entry 3
1. EDCI, BzOH, DMAP
THF, –78 °C
2 M HCl, 60 °C
15a
16b: TBSOTf
16c: BzOH
Unfolding
Inside out
16a: R = K
16b: R = TBS
16c: R = Bz
(Kb removed for clarity)
(Bz removed for clarity)

studies have laid a foundation for new, enabling chemistry—an asymmetric Reformatsky aldol, a solvent-controlled Mukaiyama hydration and a chemoselective alkyn oxidation—and a platform for the functional modification of bilobalide.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-019-1690-5.

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Data availability
All data is available in the text of this Article or its Supplementary Information. Structural parameters are available from the Cambridge Crystallographic Data Centre (CCDC) under the following reference numbers: (−)-5, CCDC 1911131; (−)-8, CCDC 1911128; 12, CCDC 1911129; and 16c, CCDC 1911127.

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Author contributions
R.A.S., M.A.B., R.M.D. and M.O. conceived the project. R.A.S. directed the research, and R.A.S., M.O., M.A.B. and R.M.D. composed the manuscript and the Supporting Information section. M.O., M.A.B. and R.M.D. completed a first-generation synthesis of rac-1. M.A.B. conceived and developed the catalytic asymmetric synthesis of (−)-7. M.A.B. observed, designed and optimized the parallel kinetic resolution of rac-9. M.O. and R.M.D. screened and optimized conditions for the alkyne oxidation of rac-12 and (−)-12. R.M.D. developed the hydration of rac-8 and (−)-8 and optimized scale-up campaigns of rac-6 and (−)-6. M.A.B. and R.M.D. conducted large-scale syntheses of rac-8 and (−)-8. M.A.B. and R.M.D. investigated the rearrangement of rac-5 and (−)-5 to 16a-c. M.O. discovered an oxidation of rac-5 to rac-1. M.A.B. investigated the rearrangement of rac-6 and (−)-6 to 16b and 16c, and discovered conditions that were utilized for the oxidation of rac-5 and (−)-5 to rac-1 and (−)-1. M.A.B. and R.M.D. both optimized this process.

Competing interests
The authors declare no competing interests.

Additional information
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Correspondence and requests for materials should be addressed to M.O. or R.A.S.

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