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Unexpected stereochemical tolerance for the biological activity of tyroscherin

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ABSTRACT

Here we describe the concise syntheses of the 15 diastereomers and key analogs of the natural product tyroscherin. While systematic analysis of the analogs clearly demonstrated that the hydrocarbon tail is important for biological activity, structure–activity relationship studies of the complete tyroscherin diastereoarray revealed a surprisingly expansive stereochemical tolerance for the cytotoxic activity. Our results represent a departure from the tenet that biological activity is constrained to a narrow pharmacophore, and highlight the recently emerging appreciation for stereochemical flexibility in defining the essential structural elements of biologically active small molecules.

1. Introduction

It is widely believed that the pharmacological properties of a biologically active small molecule are inextricably linked to both its pendant functional groupings and their relative orientations in space.[1,2] Thus, systematic alteration of structural elements and/or their stereochemistry would be expected to significantly impact the biological activity of a natural product, thereby providing useful information about its pharmacophore. Today, most new drugs require only a single enantiomer,[3] since typically only one enantiomer of a racemic mixture binds to a target protein; the other enantiomer often plays a different role, ranging from significantly reduced binding affinity for the same target protein to interactions with other non-target proteins. Nonetheless, Schreiber and coworkers have reported some tolerance for the binding of diastereomeric ligands to a common target.[4] Their study focused on peptide binding to the SH3 domain of the tyrosine kinase Src, where stereochemical variation of a single stereocenter attached to the C-terminus of a poly-proline hexamer resulted in nominal change to the dissociation constant. More recently, Breinbauer and coworkers have discovered the first example of the simultaneous (i.e., non-competitive) binding of both enantiomers of a chiral molecule to the active site of an enzyme.[5,6] Although the consequence of these stereochemical changes beyond binding affinity was not determined in these cases (i.e., their impact on the functional response was not determined), these findings could have ramifications for drug screening studies, which are commonly performed with racemic mixtures, and could also have an impact on fragment-based drug discovery.[7–9]

Tyroscherin was originally reported to inhibit insulin-like growth factor-1 (IGF-1)-induced growth of MCF-7 human breast cancer cells more potently than bovine serum (FBS)-induced growth.[10,11] The reported highly selective inhibition of IGF-1 induced growth of MCF-7 cancer cells made this molecule an interesting tool for chemical biology studies, with the only activity analyses of this natural product having been performed by the groups that originally isolated it.[11]

Two total syntheses of tyroscherin have been communicated by the Watanabe group and the Maier group.[11–13] Recently, we also reported the total synthesis and biological evaluation of tyroscherin utilizing a cross metathesis approach (Fig. 1).[14] Our aim was to correct the initially reported tyroscherin structure and to investigate its putative IGF-specificity, the latter of which we failed to confirm.

Herein, we report a concise and convergent synthesis of the 15 diastereomers and key analogs of tyroscherin. Fascinatingly, an SAR study of these tyroscherin diastereomers uncovered an unexpected and intriguing stereochemical tolerance for the shared cytotoxicity. This observation may find relevance in drug discovery programs involving the screening of optically active molecules.

2. Results and discussion

2.1. Syntheses and biological characterization of tyroscherin diastereomers

To establish extensive SAR studies and to determine the correct structure of tyroscherin, we synthesized all possible stereoisomers

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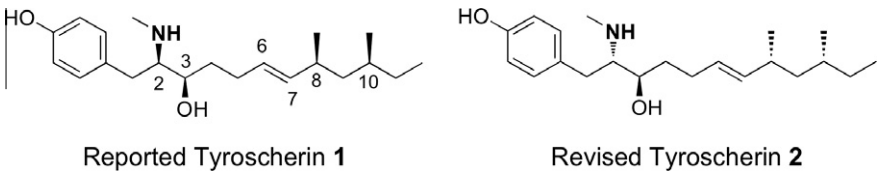


Figure 1. Structure of reported tyroscherin **1** and revised tyroscherin **2**.

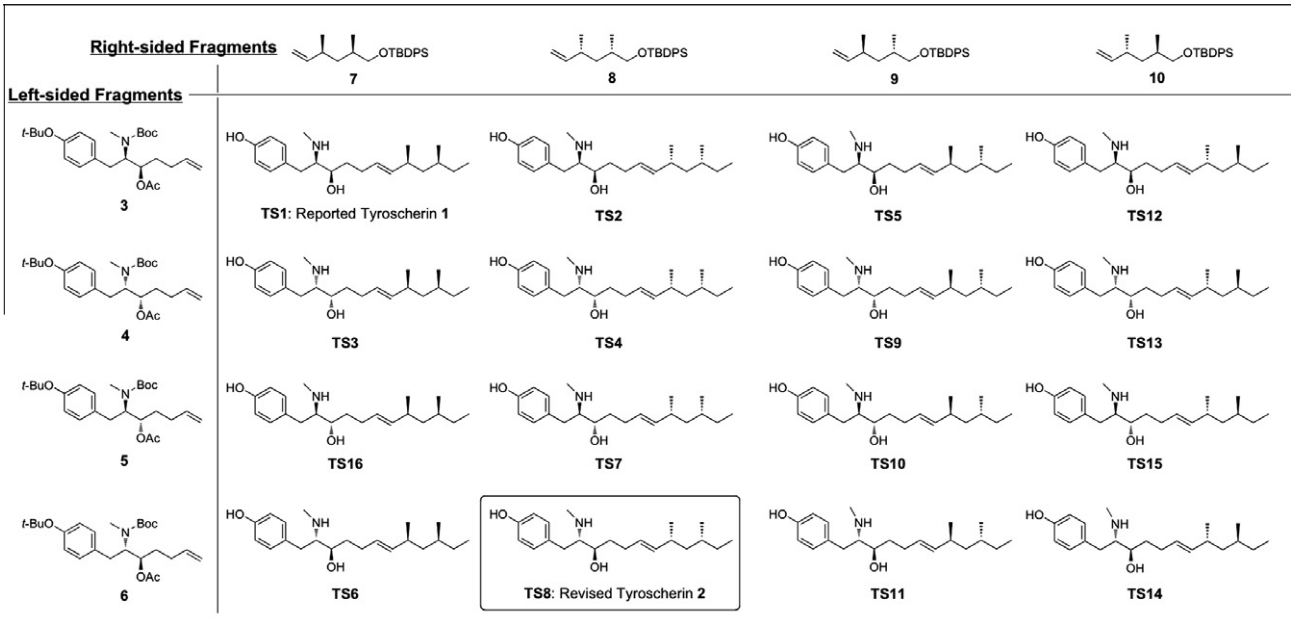


Figure 2. Synthesis of stereoarrayed 16 tyroscherin stereoisomers. TS, TyroScherin.

of **1** using a cross metathesis reaction we had employed to generate tyroscherin **1** (Fig. 2).[14] As shown in Figure 2, this was achieved with eight stereochemically different fragments: **3**, **4**, **5**, **6**, **7**, **8**, **9**, and **10**. As previously reported, **TS8** matched in all spectral and physical data to the published natural product.[14]

All diastereomers were examined for their ability to inhibit serum-driven mitogenesis versus IGF-driven mitogenesis. Surprisingly, despite the diversity of the stereochemical alterations implemented, all diastereomers had virtually identical biological activities (Table 1). Thus, while tyroscherin is a potent cytotoxic agent, we were unable to validate the claim of its enhanced potency to inhibit IGF signaling. Instead, our data support our previous findings in that tyroscherin acts as a more generalized antiproliferative agent against many growth factors.

This implies that all tyroscherin diastereomers not only bind their target with uniform affinity, but also retain the same degree of activity—that is, none of the bound diastereomers elicited a neutralized or reversed pharmacodynamic effect. Thus, the syntheses and biological characterization of all possible tyroscherin diastereomers revealed an unexpected and expansive stereochemical tolerance for the observed cytotoxic activity. While there were occasional minor differences in relative potency of inhibition between serum and IGF-driven proliferation, none of them were of the magnitude reported in the original isolation or recent structural revision report.[11,12]

Table 1
Inhibitory activity of 16 TS stereoisomers using MCF-7 cells[a]

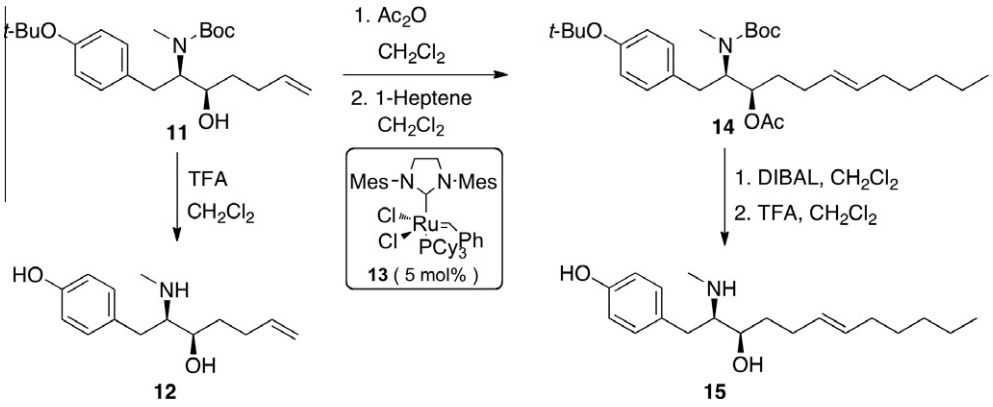
Compound	10 ng/mL IGF	IC_{50} (μM) 100 ng/mL IGF	10% FBS
TS1 (2*R*,3*R*,8*S*,10*S*)	2.07 ± 0.40	2.27 ± 0.31	4.60 ± 0.79
TS2 (2*R*,3*R*,8*R*,10*R*)	2.00 ± 0.69	1.87 ± 0.46	4.23 ± 1.00
TS3 (2*S*,3*S*,8*S*,10*S*)	1.77 ± 0.25	1.83 ± 0.25	5.40 ± 3.15
TS4 (2*S*,3*S*,8*R*,10*R*)	1.90 ± 0.44	2.27 ± 1.07	5.97 ± 2.15
TS5 (2*R*,3*R*,8*S*,10*R*)	2.07 ± 0.23	1.97 ± 0.65	4.83 ± 2.23
TS6 (2*S*,3*R*,8*S*,10*S*)	1.83 ± 0.49	2.20 ± 0.70	4.47 ± 2.90
TS7 (2*R*,3*S*,8*R*,10*R*)	3.63 ± 2.39	2.33 ± 0.25	4.93 ± 4.14
TS8 (2*S*,3*R*,8*R*,10*R*)	2.23 ± 0.74	3.07 ± 0.67	7.13 ± 2.78
TS9 (2*S*,3*S*,8*S*,10*R*)	3.10 ± 1.74	3.13 ± 1.29	6.47 ± 2.10
TS10 (2*R*,3*S*,8*S*,10*R*)	2.90 ± 1.68	2.03 ± 0.23	4.80 ± 2.34
TS11 (2*S*,3*R*,8*S*,10*R*)	2.00 ± 0.53	2.30 ± 0.36	7.03 ± 5.70
TS12 (2*R*,3*R*,8*R*,10*S*)	2.50 ± 1.04	3.07 ± 0.90	5.70 ± 6.38
TS13 (2*S*,3*S*,8*R*,10*S*)	2.76 ± 0.60	2.23 ± 0.35	7.37 ± 5.24
TS14 (2*S*,3*R*,8*R*,10*S*)	2.77 ± 1.55	2.76 ± 1.65	6.07 ± 5.19
TS15 (2*R*,3*S*,8*R*,10*S*)	2.90 ± 1.93	2.90 ± 0.98	7.67 ± 0.76
TS16 (2*R*,3*S*,8*S*,10*S*)	1.87 ± 0.31	2.73 ± 1.46	8.87 ± 1.30
Reported tyroscherin	30 nM (in serum-free medium containing IGF-1)		

[a] ^{3}H-Thymidine incorporation assay.

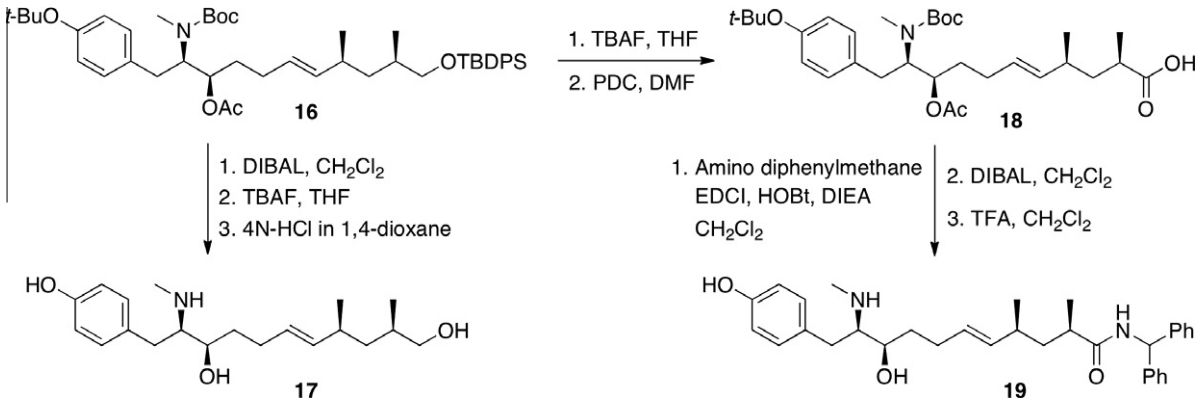
2.2. Syntheses and biological characterization of tyroscherin analogs

The surprising results concerning the conserved biological activity among the diastereomers led us to question whether the hydrocarbon tail might be unimportant for biological activity. Thus, in the next phase of our tyroscherin structure–activity relationship studies, we synthesized five structurally distinct tyroscherin analogs: **12**, **15**, **17**, **19**, and **23** according to our reported methods (Schemes 1–3).[14]

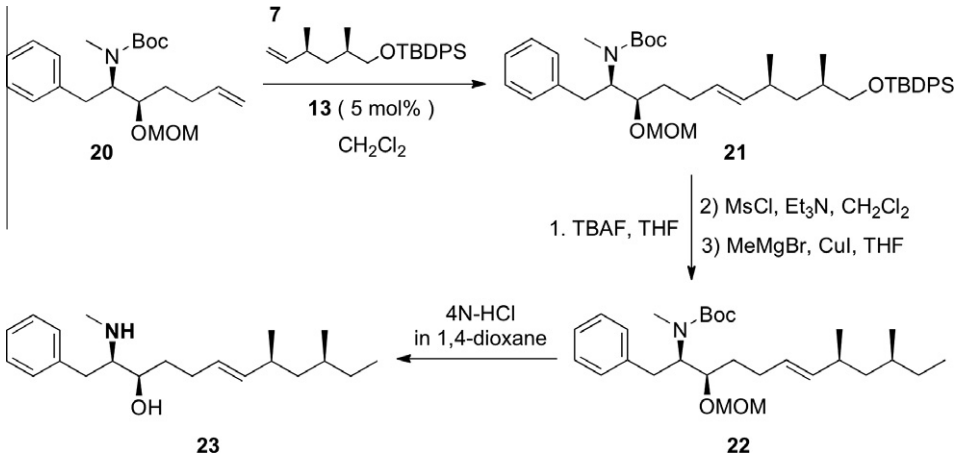
The truncated analog **12** was prepared by the treatment of **11** with TFA. Analog **15** was obtained via a four-step sequence from



Scheme 1. Synthesis of tyroscherin analogs **12** and **15**.



Scheme 2. Synthesis of tyroscherin analogs **17** and **19**.



Scheme 3. Synthesis of tyroscherin analog **23**.

alcohol **11**. Protection of hydroxyl group as an acetate and cross metathesis with 1-heptene in the presence of 5 mol % Grubbs catalyst **13** afforded exclusively the *E*-isomer of the adduct **14**.[15,16] Deprotection of acetyl in **14** with DIBAL followed by deprotection of Boc and *tert*-butyl groups with TFA gave analog **15**.

Analog **17** was achieved from the known intermediate **16**. Three sequential deprotections of **16** with DIBAL, TBAF, and 4 N-HCl produced analog **17** having a terminal hydroxyl at the right-side. Analog **19** with a bulky benzhydryl amide was obtained from TBDPS ether **16** via a five-step sequence. Treatment of ether **16** with TBAF afforded the alcohol, and oxidation of the alcohol with PDC and DMF provided acid **18**. Coupling reaction of acid **18** and amino diphenylmethane using EDCI, deprotection of acetate with DIBAL, and treatment of TFA gave analog **19**.

Scheme 3 summarizes the synthesis of analog **23** that removed the hydroxyl group from the tyrosine fragment. Cross metathesis of fragment **20** with fragment **7** produced the *E*-isomer of the adduct **21**. Treatment of TBDPS ether **21** with TBAF gave the alcohol,

Table 2
Growth inhibitory activity of TS analogs using MCF-7 cells[a]

Compound	10 ng/mL IGF	IC_{50} (μM) 100 ng/mL IGF	10% FBS
12	>100	>100	>100
15	18.2 ± 6.33	24.0 ± 6.68	19.8 ± 6.86
17	149 ± 26.7	76.5 ± 16.3	92.6 ± 18.6
19	19.1 ± 4.95	28.6 ± 6.78	13.7 ± 3.32
23	2.97 ± 1.34	2.63 ± 0.87	5.17 ± 4.89
TS8	2.23 ± 0.74	3.07 ± 0.67	7.13 ± 2.78
Reported tyroscherin	30 nM (in a serum-free medium containing IGF-1)		

[a] ^{3}H-Thymidine incorporation assay.

and mesylation of this primary alcohol followed by treatment with MeMgBr and CuI resulted in the addition of the terminal methyl group. Finally, deprotection using 4 N-HCl in 1,4-dioxane afforded analog **23**.

These analogs were then tested in the same MCF-7 cell anti-proliferation assays used to analyze tyroscherin diastereomers **TS1** through **TS16** (Table 2). Paradoxically, these analogs clearly demonstrated that the hydrocarbon tail was important for biological activity even though its stereochemistry was found to be unimportant. First, truncation of the hydrocarbon tail led to a biologically inactive molecule **12**, despite the fact that it retained the olefin and all hydrogen-bond donors/acceptors. Similarly, removal of the methyl groups at the C8 and C10 positions of **1** or the addition of a polar hydroxyl group at the right terminal led to significant reductions in the activity.

The results of analogs **12**, **15**, and **17** led to speculation that the length and/or overall hydrophobicity of the tail is important for biological activity of tyroscherin. Thus, we introduced a bulky benzhydryl amide group (**19**) to the right end of the tail: despite the steric bulk of the additional group, the hydrocarbon tail of **19** is arguably closer in length and hydrophobicity to that of **TS8** than were those of analogs **12** and **17**. Interestingly, while the activity of analog **19** was somewhat less than **TS8** itself, analog **19** was indeed far more potent than analogs **12** and **17**. Tangential to this, removal of the aromatic hydroxyl group (**23**) had no impact on activity. While it had been tempting to speculate that phosphorylation of tyroscherin at this position by a tyrosine kinase might have been key for its biological activity, the finding with analog **23** indicates this mechanism is unlikely. Taken together, the data obtained indicates that the hydrocarbon tail vis-à-vis it's overall length or degree of hydrophobicity is essential for biological activity. Further studies are therefore needed to investigate SAR studies of the revised tyroscherin.

3. Conclusions

In conclusion, our concise, unified syntheses of the 15 diastereomers and key analogs of tyroscherin afforded a comprehensive biological analysis of this natural product. Surprisingly, all diastereomers possess similar anti-proliferative activity in cell proliferation assays irrespective of whether the cells were stimulated with IGF or serum. We are able to show that this stereochemical tolerance does not extend to structural alterations in the hydrocarbon tail, indicating a role for this segment of the natural product in mediating its biological activity. The degree of tolerated stereochemical divergence shown with tyroscherin involves all four independent stereocenters, comprising the majority of the molecular structure and demonstrating a remarkable stereochemical tolerance. The extent to which removal of specific functional groups or reduction of hydrocarbon tail hydrophobicity or length can have a negative effect on activity provides strong evidence that the observed similarity in potency of all 16 diastereomers is due to a common target, rather than non-specific effects.

Our results obtained with a functional readout are a noteworthy advance over previous demonstrations of stereochemical tolerance, which were limited to measuring binding affinities without determining the biological impact of the stereochemical alterations. Moreover, this study sheds new light on the stereochemical requirements (or potential lack thereof) of biologically active natural products, and provides proof of a new type of enantiomer behavior that may have important implications for drug discovery. Efforts to identify the in vivo biological target of tyroscherin and elucidate its mode of action are currently underway.

4. Materials and methods

Eight key fragments and tyroscherin stereoisomers (**TS1**–**TS16**) were synthesized by the reported procedure (*Org. Lett.* **2010**, *12*, 4308–4311). See Supplementary data for the detailed experimental procedure.

4.1. Tyroscherin diastereomers

TS1: ^{1}H NMR (500 MHz, CD_3OD) δ 7.05 (d, J = 8.5 Hz, 2H), 6.72 (d, J = 8.5 Hz, 2H), 5.32 (dt, J = 15.3, 6.7 Hz, 1H), 5.17 (dd, J = 15.3, 8.2 Hz, 1H), 3.53 (dt, J = 9.0, 3.9 Hz, 1H), 2.77–2.72 (m, 1H), 2.72 (dd, J = 11.8, 5.6 Hz, 1H), 2.67 (dd, J = 11.8, 5.3 Hz, 1H), 2.40 (s, 3H), 2.15–2.06 (m, 2H), 2.02–1.96 (m, 1H), 1.59–1.54 (m, 1H), 1.50–1.44 (m, 1H), 1.35–1.25 (m, 2H), 1.22 (ddd, J = 13.9, 9.6, 4.6 Hz, 1H), 1.15–1.09 (m, 1H), 0.97 (ddd, J = 13.8, 9.0, 5.1 Hz, 1H), 0.90 (d, J = 6.7 Hz, 3H), 0.84 (t, J = 7.3 Hz, 3H), 0.81 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.1, 138.2, 131.2, 130.5, 129.0, 116.5, 71.2, 66.8, 45.6, 36.0, 35.7, 34.7, 34.2, 33.2, 31.1, 28.8, 22.3, 19.3, 11.6. FTIR (neat, cm^{-1}) 3239 (br), 2957, 2923, 2873, 1615, 1515, 1457, 1263, 1225. LRMS (ES+) $[M+H]^+$ 334.69, $[M+Na]^+$ 356.64. HRMS (ES+) calculated for $C_{21}H_{36}NO_2$ $[M+H]^+$ 334.2746, found 334.2741. $[\alpha]_D^{20}$ +21.5 (c 0.346, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.16 (UV, CAM).

TS2: ^{1}H NMR (500 MHz, CD_3OD) δ 7.10 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 8.5 Hz, 2H), 5.27 (dt, J = 15.2, 6.5 Hz, 1H), 5.16 (dd, J = 15.3, 8.2 Hz, 1H), 3.62 (dt, J = 8.0, 4.7 Hz, 1H), 3.14 (ddd, J = 8.6, 8.6, 2.9 Hz, 1H), 2.93 (dd, J = 14.1, 5.9 Hz, 1H), 2.85 (dd, J = 14.1, 8.4 Hz, 1H), 2.64 (s, 3H), 2.12–2.07 (m, 1H), 2.07–1.97 (m, 2H), 1.55–1.50 (m, 1H), 1.30–1.24 (m, 1H), 1.30–1.24 (m, 2H), 1.14–1.07 (m, 1H), 1.20 (ddd, J = 13.7, 9.6, 4.3 Hz, 1H), 0.96 (ddd, J = 13.9, 8.7, 5.1 Hz, 1H), 0.89 (d, J = 6.7 Hz, 3H), 0.84 (t, J = 7.3 Hz, 3H), 0.81 (d, J = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.8, 138.6, 131.3, 128.4, 127.9, 116.8, 68.9, 66.1, 45.5, 35.7, 35.3, 34.3, 33.1, 32.1, 31.1, 29.3, 22.3, 19.3, 11.6. LRMS (ES+) $[M+H]^+$ 334.25, $[M+Na]^+$ 356.21, $[\alpha]_D^{20}$ −20.1 (c 0.6, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.18 (UV, CAM).

TS3: ^{1}H NMR (500 MHz, CD_3OD) δ 7.10 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 8.5 Hz, 2H), 5.27 (dt, J = 15.3, 6.7 Hz, 1H), 5.15 (dd, J = 15.3, 8.2 Hz, 1H), 3.62 (dt, J = 8.0, 4.6 Hz, 1H), 3.16 (ddd, J = 8.5, 8.5, 5.6 Hz, 1H), 2.94 (dd, J = 14.1, 6.0 Hz, 1H), 2.86 (dd, J = 14.1, 8.5 Hz, 1H), 2.65 (s), 2.13–2.08 (m, 1H), 2.07–1.95 (m, 2H), 1.56–1.50 (m, 2H), 1.31–1.24 (m, 2H), 1.14–1.08 (m, 1H), 1.20 (ddd, J = 13.7, 9.6, 4.3 Hz, 1H), 0.95 (ddd, J = 13.6, 9.6, 5.1 Hz, 1H), 0.88 (d, J = 6.6 Hz, 3H), 0.84 (t, J = 7.3 Hz, 3H), 0.80 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.8, 138.6, 131.3, 128.4, 127.8, 116.8, 68.8, 66.1, 45.5, 35.7, 35.3, 34.2, 33.1, 32.0, 31.1, 29.3, 22.3, 19.3, 11.6. LRMS (ES+) $[M+H]^+$ 334.10, $[M+Na]^+$ 356.21, $[\alpha]_D^{20}$ +19.6 (c 0.75, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.19 (UV, CAM).

TS4: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 8.5 Hz, 2H), 5.27 (dt, J = 15.3, 6.6 Hz, 1H), 5.15 (dd, J = 15.3, 8.2 Hz, 1H), 3.63 (dt, J = 11.0, 6.3 Hz, 1H), 3.21 (ddd, J = 8.6, 8.6, 5.5 Hz, 1H), 2.96 (dd, J = 14.2, 5.8 Hz, 1H), 2.87 (dd, J = 14.2,

8.5 Hz, 1H), 2.67 (s, 3H), 2.15–2.07 (m, 1H), 2.06–1.95 (m, 2H), 1.56–1.51 (m, 2H), 1.31–1.24 (m, 2H), 1.15–1.07 (m, 1H), 1.20 (ddd, J = 13.9, 9.5, 4.6 Hz, 1H), 0.96 (ddd, J = 13.8, 8.8, 4.8 Hz, 1H), 0.89 (d, J = 6.7 Hz, 3H), 0.84 (t, J = 7.4 Hz, 3H), 0.81 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.9, 138.5, 131.4, 128.3, 127.6, 116.8, 68.7, 65.9, 44.5, 35.7, 35.4, 34.1, 33.1, 31.8, 31.1, 29.3, 22.2, 19.3, 11.6. $[\alpha]_D^{20}$ −18.6 (c 0.32, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.18 (UV, CAM).

TS5: ^{1}H NMR (500 MHz, CD_3OD) δ 7.10 (d, J = 8.4 Hz, 2H), 6.76 (d, J = 8.4 Hz, 2H), 5.28 (dt, J = 15.3, 6.4 Hz, 1H), 5.21 (dd, J = 15.4, 7.2 Hz, 1H), 3.62 (dt, J = 11.0, 6.4 Hz, 1H), 3.17 (ddd, J = 8.2, 8.2, 5.6 Hz, 1H), 2.94 (dd, J = 14.2, 5.9 Hz, 1H), 2.86 (dd, J = 14.2, 8.4 Hz, 1H), 2.65 (s, 3H), 2.13–2.07 (m, 1H), 2.06–1.97 (m, 2H), 1.56–1.51 (m, 2H), 1.37–1.30 (m, 2H), 1.17–1.12 (m, 1H), 1.10 (ddd, J = 13.3, 7.2, 5.8 Hz, 1H), 1.03 (ddd, J = 13.9, 7.1, 5.8 Hz, 1H), 0.87 (d, J = 6.7 Hz, 3H), 0.84 (t, J = 7.3 Hz, 3H), 0.82 (d, J = 6.5 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.8, 139.1, 131.4, 127.9, 127.8, 116.8, 68.8, 66.0, 45.5, 35.4, 35.3, 34.2, 33.0, 32.0, 30.2, 29.3, 21.2, 19.8, 11.5. $[\alpha]_D^{20}$ −2.3 (c 0.9, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.16 (UV, CAM).

TS6: ^{1}H NMR (500 MHz, CD_3OD) δ 7.11 (d, J = 8.4 Hz, 2H), 6.77 (d, J = 8.4 Hz, 2H), 5.35 (dt, J = 15.1, 6.8 Hz, 1H), 5.22 (dd, J = 15.2 Hz, 8.1, 1H), 3.84 (dt, J = 8.9, 3.7 Hz, 1H), 3.35 (ddd, J = 7.6, 7.6, 3.5 Hz, 1H), 2.93 (dd, J = 14.7, 6.7 Hz, 1H), 2.85 (dd, J = 14.7, 6.7 Hz, 1H), 2.62 (s, 3H), 2.22–2.11 (m, 2H), 2.03–1.95 (m, 1H), 1.57–1.48 (m, 2H), 1.35–1.27 (m, 2H), 1.15–1.09 (m, 1H), 1.23 (ddd, J = 14.0, 9.5, 4.6 Hz, 1H), 0.99 (ddd, J = 13.9, 8.8, 4.8 Hz, 1H), 0.92 (d, J = 6.7 Hz, 3H), 0.85 (t, J = 7.3 Hz, 3H), 0.81 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.9, 138.6, 131.2, 128.4, 127.6, 116.9, 68.7, 66.6, 45.5, 35.7, 33.2, 33.1, 32.4, 32.3, 31.1, 29.8, 22.3, 19.3, 11.6. $[\alpha]_D^{20}$ +19.7 (c 0.76, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.18 (UV, CAM).

TS7: ^{1}H NMR (400 MHz, CD_3OD) δ 7.10 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 8.5 Hz, 2H), 5.35 (dt, J = 15.2, 6.8 Hz, 1H), 5.22 (dd, J = 15.2, 8.1 Hz, 1H), 3.82 (dt, J = 8.8, 3.1 Hz, 1H), 3.27 (ddd, J = 7.1, 7.1, 2.8 Hz, 1H), 2.90 (dd, J = 14.6, 6.7 Hz, 1H), 2.82 (dd, J = 14.6, 6.7 Hz, 1H), 2.58 (s, 3H), 2.24–2.12 (m, 2H), 2.04–1.94 (m, 1H), 1.57–1.48 (m, 2H), 1.34–1.27 (m, 2H), 1.16–1.09 (m, 1H), 1.23 (ddd, J = 14.0, 9.4, 4.6 Hz, 1H), 0.99 (ddd, J = 13.8, 8.8, 4.5 Hz, 1H), 0.92 (d, J = 6.7 Hz, 3H), 0.85 (t, J = 7.3 Hz, 3H), 0.81 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 157.8, 138.6, 131.2, 128.5, 128.0, 116.8, 69.0, 66.7, 45.5, 35.7, 33.2, 33.1, 32.7, 32.6, 31.1, 29.9, 22.3, 19.3, 11.7. $[\alpha]_D^{20}$ −19.2 (c 0.48, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.18 (UV, CAM).

TS8: ^{1}H NMR (500 MHz, CD_3OD) δ 7.11 (d, J = 8.3 Hz, 2H), 6.77 (d, J = 8.3 Hz, 2H), 5.33 (dt, J = 15.3, 6.7 Hz, 1H), 5.21 (dd, J = 15.3, 8.2 Hz, 1H), 3.83 (dt, J = 9.2, 3.1 Hz, 1H), 3.34 (ddd, J = 7.5, 7.5, 2.6 Hz, 1H), 2.91 (dd, J = 14.7, 7.0 Hz, 1H), 2.86 (dd, J = 14.7, 7.7 Hz, 1H), 2.62 (s, 3H), 2.23–2.12 (m, 2H), 1.99 (m, 1H), 1.59–1.45 (m, 2H), 1.22 (ddd, J = 13.8, 9.5, 4.5 Hz, 1H), 1.12 (m, 1H), 0.99 (ddd, J = 13.6, 8.9, 4.9 Hz, 1H), 0.91 (d, J = 6.6 Hz, 3H), 0.85 (t, J = 7.4 Hz, 3H), 0.82 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.9, 138.8, 131.2, 128.4, 127.6, 116.9, 68.7, 66.7, 45.5, 35.8, 33.2, 33.0, 32.4, 32.3, 31.1, 29.8, 22.3, 19.3, 11.7. LRMS (ES+) $[M+H]^+$ 334.67, $[M+Na]^+$ 356.62. HRMS (ES+) calculated for $C_{21}H_{36}NO_2$ $[M+H]^+$ 334.2748, found 334.2742. $[\alpha]_D^{20}$ −19.7 (c 0.483, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.17 (UV, CAM).

TS9: ^{1}H NMR (500 MHz, CD_3OD) δ 7.10 (d, J = 8.2 Hz, 2H), 6.76 (d, J = 8.1 Hz, 2H), 5.26 (dt, J = 15.0, 6.3 Hz, 1H), 5.21 (dd, J = 15.1, 7.1 Hz, 1H), 3.62 (dt, J = 8.6, 3.8 Hz, 1H), 3.17 (ddd, J = 8.7, 8.7, 5.1 Hz, 1H), 2.94 (dd, J = 14.2, 5.8 Hz, 1H), 2.86 (dd, J = 14.2, 8.5 Hz, 1H), 2.66 (s, 3H), 2.13–2.07 (m, 1H), 2.07–2.02 (m, 1H), 2.03–1.95 (m, 1H), 1.56–1.50 (m, 2H), 1.38–1.32 (m, 2H), 1.11–1.06 (m, 1H), 1.15 (ddd, J = 13.6, 6.9, 6.9 Hz, 1H), 1.02 (ddd, J = 13.9, 6.8, 6.8 Hz, 1H), 0.87 (d, J = 6.8 Hz, 3H), 0.84 (t, J = 7.1 Hz, 3H), 0.82 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.8, 139.1, 131.4, 127.9, 127.8, 116.8, 68.7, 66.0, 45.5, 35.4, 35.3, 34.2, 33.0, 32.0, 30.2, 29.3, 21.2, 19.8, 11.5. $[\alpha]_D^{20}$ +7.9 (c 0.88, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.18 (UV, CAM).

TS10: ^{1}H NMR (500 MHz, CD_3OD) δ 7.10 (d, J = 8.3 Hz, 2H), 6.76 (d, J = 8.2 Hz, 2H), 5.34 (dt, J = 15.3, 6.3 Hz, 1H), 5.28 (dd, J = 15.3, 7.2 Hz, 1H), 3.80 (dt, J = 9.3, 3.1 Hz, 1H), 3.24 (ddd, J = 7.2, 7.2, 2.6 Hz, 1H), 2.87 (dd, J = 14.7, 7.9 Hz, 1H), 2.82 (dd, J = 14.6, 6.9 Hz, 1H), 2.58 (s, 3H), 2.23–2.12 (m, 2H), 2.03–1.95 (m, 1H), 1.59–1.45 (m, 2H), 1.39–1.32 (m, 2H), 1.12–1.08 (m, 1H), 1.17 (ddd, J = 13.5, 6.7, 6.7 Hz, 1H), 1.08–1.02 (m, 1H), 0.89 (d, J = 6.7 Hz, 3H), 0.85 (t, J = 7.3 Hz, 3H), 0.83 (d, J = 6.1 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.8, 139.2, 131.2, 128.2, 128.0, 116.8, 69.0, 66.8, 45.6, 35.5, 33.1, 33.0, 32.8, 32.7, 30.2, 29.9, 21.2, 19.8, 11.5. $[\alpha]_D^{20}$ +3.0 (c 0.307, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.18 (UV, CAM).

TS11: ^{1}H NMR (500 MHz, CD_3OD) δ 7.09 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 8.5 Hz, 2H), 5.35 (dt, J = 15.3, 6.3 Hz, 1H), 5.28 (dd, J = 15.3, 7.1 Hz, 1H), 3.79 (dt, J = 9.0, 3.3 Hz, 1H), 3.19 (ddd, J = 7.5, 7.5, 2.7 Hz, 1H), 2.87 (dd, J = 14.7, 6.7 Hz, 1H), 2.79 (dd, J = 14.7, 7.8 Hz, 1H), 2.55 (s, 3H), 2.23–2.11 (m, 2H), 2.04–1.95 (m, 1H), 1.60–1.45 (m, 2H), 1.39–1.31 (m, 2H), 1.11–1.07 (m, 1H), 1.17 (ddd, J = 13.4, 13.4, 6.8 Hz, 1H), 1.07–1.02 (m, 1H), 0.91 (d, J = 6.7 Hz, 3H), 0.85 (t, J = 7.3 Hz, 3H), 0.84 (d, J = 6.5 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.7, 139.1, 131.2, 128.4, 128.0, 116.8, 69.2, 66.8, 45.5, 35.5, 33.1, 33.0, 33.0, 32.9, 30.2, 29.9, 21.2, 19.8, 11.5. $[\alpha]_D^{20}$ +0.4 (c 0.32, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.17 (UV, CAM).

TS12: ^{1}H NMR (500 MHz, CD_3OD) δ 7.10 (d, J = 8.4 Hz, 2H), 6.76 (d, J = 8.4 Hz, 2H), 5.26 (dt, J = 15.3, 6.3 Hz, 1H), 5.21 (dd, J = 15.2, 7.2 Hz, 1H), 3.62 (dt, J = 8.0, 4.7 Hz, 1H), 3.16 (ddd, J = 8.6, 8.6, 5.3 Hz, 1H), 2.94 (dd, J = 14.2, 5.8 Hz, 1H), 2.86 (dd, J = 14.2, 8.5 Hz, 1H), 2.66 (s, 3H), 2.13–2.07 (m, 1H), 2.07–2.02 (m, 1H), 2.03–1.95 (m, 1H), 1.56–1.50 (m, 2H), 1.38–1.31 (m, 2H), 1.11–1.05 (m, 1H), 1.15 (ddd, J = 13.6, 6.9, 6.9 Hz, 1H), 1.02 (ddd, J = 13.7, 7.0, 7.0 Hz, 1H), 0.87 (d, J = 6.8 Hz, 3H), 0.84 (t, J = 7.1 Hz, 3H), 0.82 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.8, 139.1, 131.4, 127.9, 127.8, 116.8, 68.7, 66.0, 45.5, 35.4, 35.3, 34.2, 33.0, 32.0, 30.2, 29.3, 21.2, 19.8, 11.5. $[\alpha]_D^{20}$ −8.3 (c 0.38, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.17 (UV, CAM).

TS13: ^{1}H NMR (500 MHz, CD_3OD) δ 7.10 (d, J = 8.4 Hz, 2H), 6.76 (d, J = 8.4 Hz, 2H), 5.27 (dt, J = 15.3, 6.3 Hz, 1H), 5.21 (dd, J = 15.4, 7.3 Hz, 1H), 3.61 (dt, J = 11.1, 6.3 Hz, 1H), 3.14 (ddd, J = 7.5, 7.5, 5.4 Hz, 1H), 2.93 (dd, J = 14.1, 5.9 Hz, 1H), 2.85 (dd, J = 14.1, 8.4 Hz, 1H), 2.64 (s, 3H), 2.13–2.07 (m, 1H), 2.06–1.95 (m, 2H), 1.56–1.51 (m, 2H), 1.37–1.30 (m, 2H), 1.17–1.12 (m, 1H), 1.10 (ddd, J = 13.3, 7.2, 5.8 Hz, 1H), 1.03 (ddd, J = 13.9, 7.1, 7.1 Hz, 1H), 0.87 (d, J = 6.7 Hz, 3H), 0.84 (t, J = 7.3 Hz, 3H), 0.82 (d, J = 6.5 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.8, 139.1, 131.4, 128.0, 127.9, 116.8, 69.0, 66.0, 45.5, 35.4, 35.3, 34.3, 33.0, 32.1, 30.2, 29.3, 21.2, 19.8, 11.5. $[\alpha]_D^{20}$ +2.1 (c 0.5, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.18 (UV, CAM).

TS14: ^{1}H NMR (500 MHz, CD_3OD) δ 7.11 (d, J = 8.3 Hz, 2H), 6.77 (d, J = 8.4 Hz, 2H), 5.34 (dt, J = 15.2, 6.4 Hz, 1H), 5.28 (dd, J = 15.2, 7.2 Hz, 1H), 3.83 (dt, J = 9.1, 3.1 Hz, 1H), 3.35 (ddd, J = 7.3, 7.3, 2.7 Hz, 1H), 2.92 (dd, J = 14.6, 7.0 Hz, 1H), 2.86 (dd, J = 14.6, 7.0 Hz, 1H), 2.62 (s, 3H), 2.22–2.12 (m, 2H), 2.03–1.95 (m, 1H), 1.58–1.45 (m, 2H), 1.39–1.32 (m, 2H), 1.12–1.08 (m, 1H), 1.17 (ddd, J = 13.6, 6.8, 6.8 Hz, 1H), 1.08–1.02 (m, 1H), 0.89 (d, J = 6.7 Hz, 3H), 0.85 (t, J = 7.3 Hz, 3H), 0.83 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.9, 139.2, 131.2, 127.9, 127.7, 116.8, 68.7, 66.7, 45.5, 35.5, 33.1, 33.0, 32.5, 32.4, 30.2, 29.8, 21.2, 19.8, 11.5. $[\alpha]_D^{20}$ −3.1 (c 1.0, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.19 (UV, CAM).

TS15: ^{1}H NMR (500 MHz, CD_3OD) δ 7.11 (d, J = 8.4 Hz, 2H), 6.77 (d, J = 8.4 Hz, 2H), 5.35 (dt, J = 15.4, 6.3 Hz, 1H), 5.28 (dd, J = 15.4, 7.3 Hz, 1H), 3.84 (dt, J = 8.9, 3.5 Hz, 1H), 3.35 (ddd, J = 7.1, 7.1,

2.5 Hz, 1H), 2.93 (dd, J = 14.7, 6.7 Hz, 1H), 2.85 (dd, J = 14.7, 7.8 Hz, 1H), 2.62 (s, 3H), 2.21–2.12 (m, 2H), 2.03–1.95 (m, 1H), 1.58–1.46 (m, 2H), 1.40–1.31 (m, 2H), 1.12–1.08 (m, 1H), 1.18 (ddd, J = 13.5, 6.7, 6.7 Hz, 1H), 1.07–1.02 (m, 1H), 0.91 (d, J = 6.7 Hz, 3H), 0.85 (t, J = 7.3 Hz, 3H), 0.84 (d, J = 6.4 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.9, 139.2, 131.2, 127.9, 127.7, 116.8, 68.7, 66.6, 45.5, 35.5, 33.2, 33.0, 32.4, 32.3, 30.2, 29.8, 21.2, 19.8, 11.5. $[\alpha]_D^{20}$ −0.2 (c 1.0, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.18 (UV, CAM).

TS16: ^{1}H NMR (500 MHz, CD_3OD) δ 7.10 (d, J = 8.4 Hz, 2H), 6.76 (d, J = 8.3 Hz, 2H), 5.34 (dt, J = 15.2, 6.7 Hz, 1H), 5.21 (dd, J = 15.3, 8.1 Hz, 1H), 3.81 (dt, J = 9.4, 3.0 Hz, 1H), 3.27 (ddd, J = 7.2, 7.2, 2.5 Hz, 1H), 2.89 (dd, J = 14.6, 6.9 Hz, 1H), 2.83 (dd, J = 14.6, 7.8 Hz, 1H), 2.59 (s, 3H), 2.23–2.12 (m, 2H), 2.03–1.95 (m, 1H), 1.59–1.52 (m, 1H), 1.51–1.45 (m, 1H), 1.34–1.26 (m, 2H), 1.15–1.09 (m, 1H), 1.23 (ddd, J = 14.0, 9.5, 4.6 Hz, 1H), 0.99 (ddd, J = 13.6, 8.7, 4.9 Hz, 1H), 0.91 (d, J = 6.6 Hz, 3H), 0.85 (t, J = 7.4 Hz, 3H), 0.82 (d, J = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 157.8, 138.7, 131.2, 128.5, 128.0, 116.8, 68.9, 66.9, 45.5, 35.8, 33.2, 33.0, 32.7, 32.6, 31.1, 29.9, 22.3, 19.3, 11.6. $[\alpha]_D^{20}$ +19.8 (c 0.42, MeOH). TLC (10% CH_3OH in CH_2Cl_2), R_f 0.17 (UV, CAM).

4.2. Cell culture

MCF-7 cells were a generous gift from Anton Bennett (Yale University, New Haven, CT) and were routinely grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum. All culture medium was supplemented with 100 units/mL penicillin G and 100 μg/mL streptomycin sulfate. Cells were grown at 37 °C in an atmosphere of 5% CO_2.

4.3. [^{3}H]-Thymidine incorporation assay

MCF-7 cells were seeded into 96 well plates in reduced serum (1%) medium at a density of 10,000 cells/well. After an overnight incubation to allow for cell attachment, the growth medium was removed and replaced with fresh serum-free medium. After at least 48 h of serum starvation to growth arrest the cells, each well was overlaid with an equal volume of medium containing double-strength mitogen and/or diastereomer. The cells were incubated another 24 h at which point each well of cells received another 20 μL of (serum-free) medium containing 2 μCi of [^{3}H]-thymidine (Perkin–Elmer, Boston, MA). Following another 4 h, the cells were harvested from the wells and passed through glass fiber filters using a Ska-Tron cell harvester (Molecular Devices, Sunnyvale, CA). The filters were transferred to vials, scintillant was added and the amount of radioactivity incorporated into the cells in the filters was quantified by scintillation counting. The resulting data was analyzed using PRISM software (GRAPHPAD Software, San Diego, CA).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.027.

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