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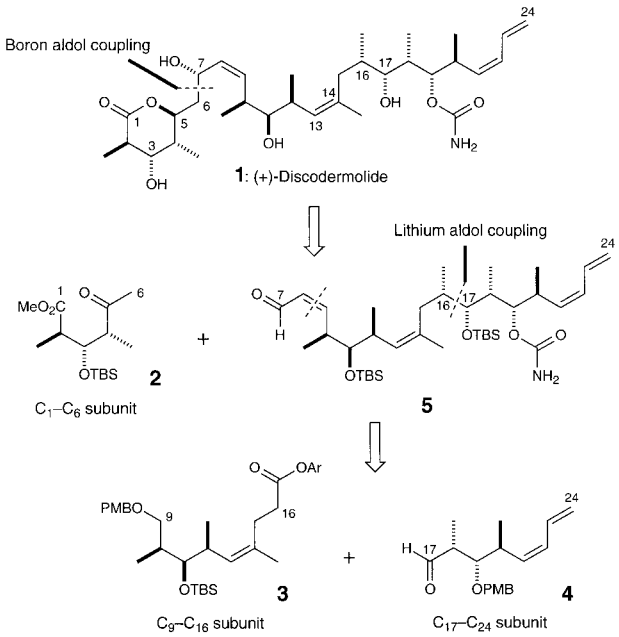

Scheme 1. Retrosynthetic analysis. Ar = 2,6-dimethylphenyl.

Total Synthesis of the Antimicrotubule Agent (+)-Discodermolide Using Boron-Mediated Aldol Reactions of Chiral Ketones**

Ian Paterson,* Gordon J. Florence, Kai Gerlach, and Jeremy P. Scott

Discodermolide (**1**) is a unique polyketide isolated in 1990 by Gunasekera et al. at the Harbor Branch Oceanographic Institute, Florida, from the Caribbean sponge *Discodermia dissoluta*.[1] Structurally (Scheme 1), it bears 13 stereogenic centers, a tetrasubstituted δ-lactone (C_1-C_5), one di- and one tri-substituted *Z*-alkene, a carbamate moiety, and a terminal *Z*-diene. Discodermolide displays potent activity as an antimitotic agent, with a similar mechanism of action to taxol (paclitaxel), namely by stabilizing microtubules and promoting the polymerization of tubulin.[2] It inhibits the growth of human breast cancer cells in vitro, as well as paclitaxel-resistant ovarian and colon cancer cells, and other multidrug resistant cells.[3] Hence, discodermolide is a particularly promising candidate for development in cancer chemotherapy, but its use is severely limited by its scarce supply (0.002% w/w isolation yield)[1] from the rare sponge source.

To date, two total syntheses of the natural (+)-discodermolide and three syntheses of the antipodal (−)-discodermolide have been reported,[4] together with various synthetic approaches.[5] Schreiber and co-workers[4a] have synthesized both antipodes and established the absolute configuration, as well as preparing a number of structural analogues.[4b] Despite these impressive efforts, there is still a pressing demand for developing a more practical and efficient synthetic route to (+)-discodermolide.

Herein, we describe a highly convergent total synthesis, which has the potential to provide useful quantities of (+)-discodermolide. Notably, our route is entirely different from earlier syntheses and is based on a novel aldol-coupling strategy, which also employs aldol reactions of chiral ketones to construct the three key subunits **2**, **3**, and **4** (Scheme 1). Our retrosynthetic analysis is based on a C_6-C_7 disconnection which leads back to the (C_1-C_6) methyl ketone **2** and the enal **5**. Further disassembly of **5** gives the (C_9-C_{16}) ester **3** and the (C_{17}-C_{24}) aldehyde **4**.

As shown in Scheme 2 the synthesis of the C_1-C_6 subunit **2** began with a boron-mediated *anti*-selective aldol reaction between the readily available[6] ethyl ketone (*S*)-**6** and acetaldehyde. The intermediate aldolate was reduced in situ with $LiBH_4$ to give diol **7** (98%, >97% diastereoselectivity (ds)).[6a] Protection as the bis-*tert*-butyldimethylsilyl (TBS) ether **8**, followed by methanolysis using catalytic CSA in $MeOH/CH_2Cl_2$ at 0 °C, gave C_5-alcohol **9** in 70% yield, together with starting material and diol **7**, which were recycled accordingly. After debenzylation, diol **10** was converted into subunit **2** by sequential double Swern oxidation, $NaClO_2$ oxidation, and esterification. This three-step operation was carried out without chromatography in 93% yield. The

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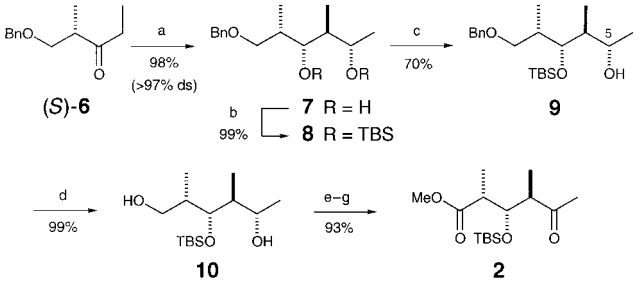
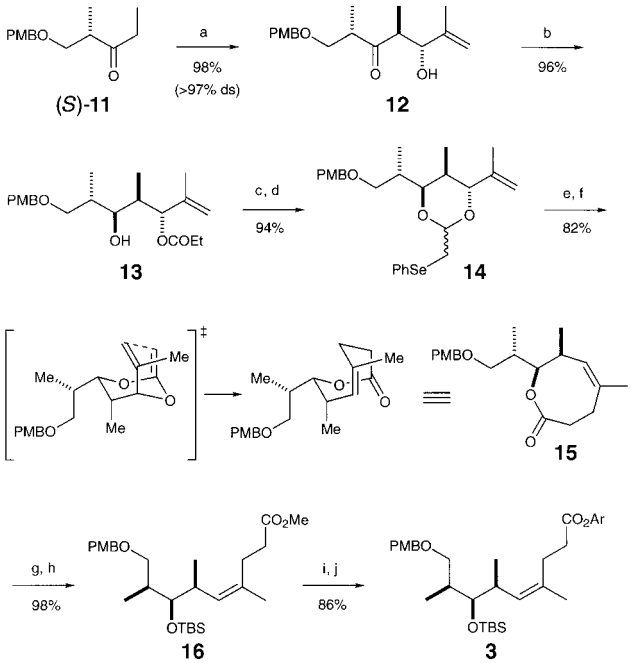


Scheme 2. Synthesis of the C_1-C_6 subunit (**2**). a) 1) $(c\mathrm{Hex})_2\mathrm{BCl}$, Et_3N, Et_2O, 0 °C, 3 h; MeCHO, −78 → −27 °C, 16 h; 2) $LiBH_4$, −78 °C, 3 h; 3) H_2O_2(30 % aq)/MeOH, NaOH(10 % aq), 0 °C, 2 h; b) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 2 h; c) CSA, MeOH/CH_2Cl_2, 0 °C, 8 h; d) 20 % $Pd(OH)_2$/C, H_2, EtOH, 20 °C, 20 h; e) 1) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, 1.5 h; 2) Et_3N, −78 → −20 °C, 20 min; f) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH, H_2O, 20 °C, 2 h; g) CH_2N_2, Et_2O, 20 °C, 5 min. Bn = benzyl, *c*Hex = cyclohexyl, TBSOTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate, CSA = camphorsulfonic acid.

synthesis can be conveniently performed on a multigram scale with the overall yield of **2** being 62 % over the seven steps.

It was of special interest to have a solution in hand to establish the *Z*-configuration of the C_{13}-C_{14} trisubstituted alkene in discodermolide with complete selectivity (Scheme 3). Commonly used methods such as Wittig olefinations give varying degrees of selectivity. On the basis of the work of Holmes et al., a Claisen-type ring expansion was used advantageously to achieve this alkene substitution pattern

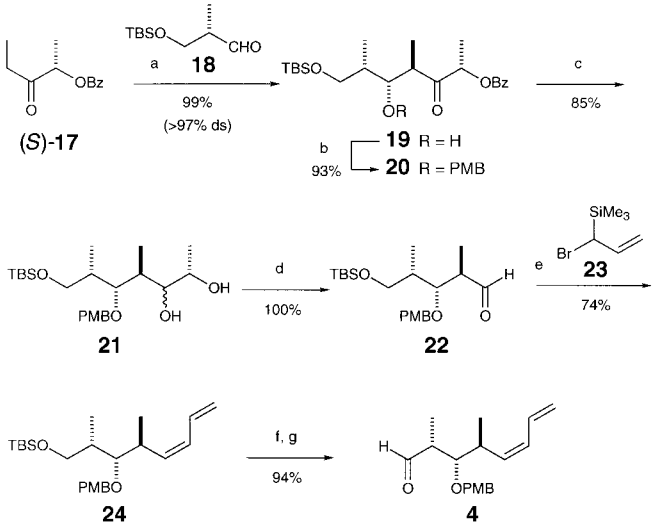


Scheme 3. Synthesis of the C_9-C_{16} subunit (**3**). a) 1) $(c\mathrm{Hex})_2\mathrm{BCl}$, Et_3N, Et_2O, 3 h; CH_2=C(Me)CHO, −78 → −27 °C, 16 h; 2) H_2O_2(30 % aq)/MeOH, pH 7 buffer, 0 °C, 2 h; b) SmI_2, EtCHO, THF, −10 °C, 2.5 h; c) K_2CO_3, MeOH, 20 °C, 3 h; d) $PhSeCH_2CH(OEt)_2$, toluene, cat. PPTS, reflux, 4.5 h; e) $NaIO_4$, $NaHCO_3$, MeOH/H_2O, 20 °C, 2 h; f) DBU, CH_2=C(OMe)OTBS, xylenes, reflux, 6 h; g) NaOMe, MeOH, 0 °C, 1 h; h) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 1.5 h; i) KOH(1 M aq), MeOH, reflux, 1 h; j) 2,6-dimethylphenol, DCC, 4-DMAP, CH_2Cl_2, 20 °C, 16 h. PPTS = pyridinium *para*-toluenesulfonate, DBU = 1,8-diazabicyclo[5.4.0]-undec-7-ene, DCC = *N*,*N*′-dicyclohexylcarbodiimide, 4-DMAP = 4-*N*,*N*-dimethylaminopyridine.

and simultaneously introduce a carbonyl group attached to C_{16}, to enable a later aldol coupling.[7] The synthesis of the aryl ester **3** started from the ethyl ketone (*S*)-**11** (that is, the *para*-methoxybenzyl (PMB) equivalent of (*S*)-**6**; available in 3 steps and 80 % yield from methyl (*S*)-3-hydroxy-2-methylpropionate).[8] Using our standard conditions,[6b,c] the boron-mediated *anti*-selective aldol reaction between (*S*)-**11** and methacrolein gave adduct **12** in high diastereoselectivity (> 97 % ds) and 99 % yield.

An Evans–Tishchenko reduction with SmI_2 and propionaldehyde formed exclusively the corresponding 1,3-*anti*-diol (96 %) protected as the mono-propionate **13**.[9] Saponification with K_2CO_3 in MeOH, followed by transacetalization with phenylselenyl acetaldehyde diethyl acetal,[10] provided a mixture of diastereomeric acetals **14** in 94 % yield, which were oxidized with $NaIO_4$ and submitted directly to the rearrangement conditions. Elimination of the selenoxide took place in refluxing xylenes (0.01 M) in the presence of DBU giving an intermediate ketene acetal, which underwent Claisen rearrangement smoothly to give the lactone **15** in 82 % yield together with 10 % of the recyclable seleno acetals **14**. The exclusive formation of the *Z*-alkene can be attributed to the preferred chairlike transition state shown in Scheme 3. Next, the 8-membered lactone **15** was converted in a four-step sequence (85 % overall) by methanolysis and TBS protection of the resultant hydroxy ester into **16**, followed by saponification and esterification with 2,6-dimethylphenol by using Steglich's protocol,[11] to give the required subunit **3** (61 % overall yield for 9 steps on a multigram scale).

The preparation of the remaining C_{17}-C_{24} subunit **4** is shown in Scheme 4. The boron-mediated aldol reaction between the lactate-derived ketone (*S*)-**17** and aldehyde **18** gave the corresponding adduct **19** in excellent yield and with *anti* selectivity (99 %, > 97 % ds).[12] Protection as the PMB ether



Scheme 4. Synthesis of the C_{17}-C_{24} subunit (**4**). a) 1) $(c\mathrm{Hex})_2\mathrm{BCl}$, Et_3N, Et_2O, 3 h; **18**, −78 → −27 °C, 16 h; 2) H_2O_2(30 % aq)/MeOH, 0 °C, 1 h; b) PMBTCA, cat. TfOH, THF, 20 °C, 9 h; c) $LiAlH_4$, THF −78 → −27 °C, 3 h; d) $NaIO_4$, MeOH, 20 °C, 30 min; e) 1) $CrCl_2$, THF, 20 °C, 16 h; 2) KH, THF, 0 °C, 1.5 h; f) CSA (0.2 equiv), MeOH/CH_2Cl_2, 20 °C, 5 h; g) DMP, CH_2Cl_2, 20 °C, 15 min. Bz = benzoyl, PMBTCA = *para*-methoxybenzyl trichloroacetimidate, TfOH = trifluoromethanesulfonic acid, DMP = Dess–Martin periodinane.

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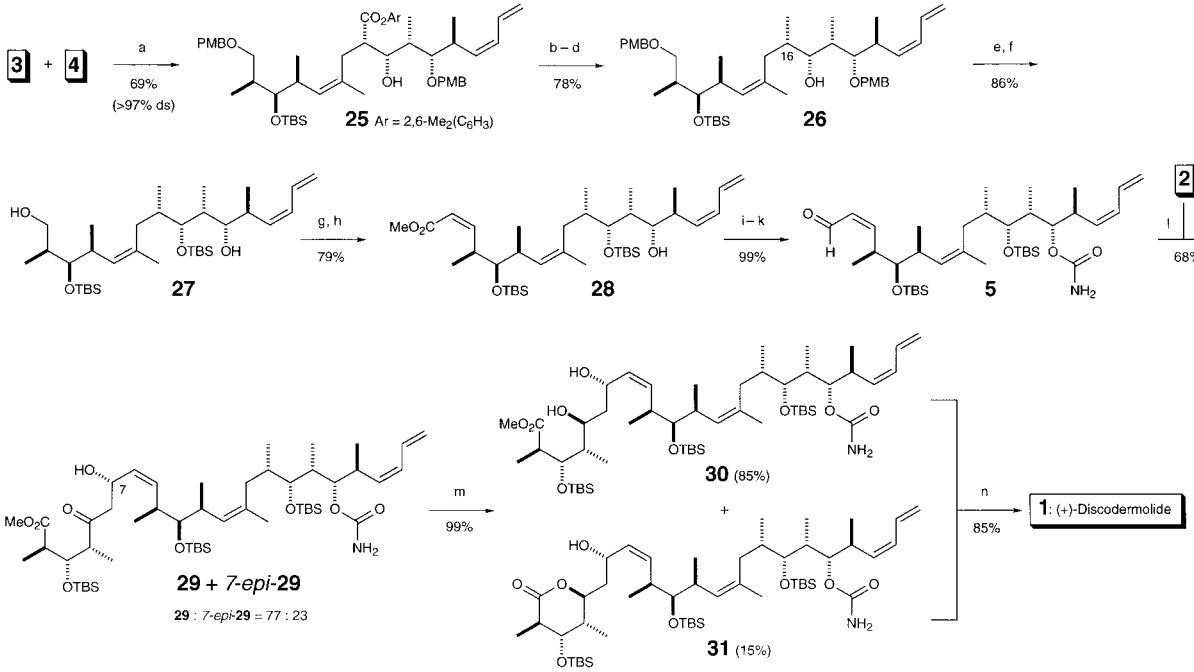
under acidic conditions gave **20**, which was reduced with $LiAlH_4$ to provide diols **21**. $NaIO_4$-mediated oxidative cleavage gave the aldehyde **22** in preparation for the introduction of the *Z*-diene moiety. This sequence was achieved in a two-step procedure, following our previously established protocol by using a Nozaki–Hiyama reaction.[5f] Addition of the aldehyde **22** and allylic bromide **23** to a suspension of $CrCl_2$ in THF produced an intermediate β-hydroxy silane, which on treatment with KH underwent a Peterson-type *syn* elimination to generate the required *Z*-diene **24** in 74% yield ($Z/E > 98:2$). Deprotection with CSA in $MeOH/CH_2Cl_2$ (94%), followed by Dess–Martin oxidation, gave aldehyde **4**. The overall yield of **4** is 54% for 8 steps carried out on a multigram scale.

Now, having all three major subunits in hand, we turned to coupling these together while at the same time installing the three stereogenic centers at C_7, C_{16}, and C_{17} (Scheme 5). The Heathcock-type ester **3** was enolized with LiTMP at $-100\,^\circ C$ to give exclusively the *E*-enolate.[13] The subsequent *anti*-selective aldol reaction with the C_{17}-C_{24} aldehyde **4** gave the Felkin–Anh adduct **25** in 69% yield and high diastereoselectivity (>97% ds). A three-step sequence of reduction with $LiAlH_4$, selective sulfonation in the presence of the secondary C_{19}-alcohol, followed by an additional $LiAlH_4$ reduction, gave the deoxygenated product **26** with the C_{16}-methyl group in place (78% yield; all reactions gave 95–100% yield based on recovered starting materials). Protection of the remaining alcohol with a TBS group and a double deprotection with DDQ provided the diol **27** (86% yield). Selective oxidation of the primary alcohol in the presence of the secondary C_{19}-alcohol was achieved using TEMPO in CH_2Cl_2 at $20\,^\circ C$.[14] The crude aldehyde obtained was converted directly into the *Z*-enoate **28** by using the Still modification of the Horner-Wadsworth-Emmons procedure (79% yield for two steps).[15] At this stage, the carbamate moiety was installed following a modification of the Kocovsky protocol.[4e, 16] A chemoselective reduction using DIBAL-H at $-78\,^\circ C$ and subsequent Dess–Martin oxidation produced the enal **5**.

The final coupling step between methyl ketone **2** and aldehyde **5** required the correct introduction of the C_7-stereocenter. Model studies had revealed that boron-mediated aldol reactions with **2** and γ-chiral *Z*-enals, related structurally to **5**, gave unexpectedly high levels of 1,4-stereoinduction from the aldehyde component in the *undesired* sense.[17] Gratifyingly, the inherent facial bias of the *Z*-enal **5** was overturned by carrying out this boron aldol coupling under reagent control using (+)-Ipc_2BCl to give 77% diastereoselectivity in favor of the desired (7*S*)-adduct **29** (52%).[18] Notably, this is the first case in which this reagent system has been used successfully in a triple asymmetric induction situation to achieve the desired stereocontrol in a mismatched aldol coupling.[19] A hydroxyl-directed reduction[20] of **29** with $Me_4NBH(OAc)_3$ provided the 1,3-*anti*-diol **30** together with pre-lactonized **31** in quantitative combined yield. Finally, global deprotection and concomitant lactonization was achieved in one pot by reaction of **30** and **31** with HF·py in THF to provide (+)-discodermolide (**1**) in 85% yield. The spectroscopic data of the synthetic material were in



Scheme 5. Subunit coupling and total synthesis of (+)-discodermolide (**1**). a) **3**, LiTMP, LiBr, THF, $-100\,^\circ C$; **4**, 7 min; b) $LiAlH_4$, THF, $-30\,^\circ C$, 3 h; c) 2,4,6-$Me_3(C_6H_2)SO_2Cl$, Et_3N, CH_2Cl_2, $20\,^\circ C$, 24 h; d) $LiAlH_4$, THF, $-10\,^\circ C$, 3 h; e) TBSOTf, Et_3N, CH_2Cl_2, $20\,^\circ C$, 24 h; f) DDQ, CH_2Cl_2/pH7 buffer, $20\,^\circ C$, 16 h; g) cat. TEMPO, BAIB, CH_2Cl_2, $20\,^\circ C$, 4h; h) $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, [18]c-6, K_2CO_3, PhMe, $-20 \rightarrow 0\,^\circ C$, 2 h; i) 1) $Cl_3CC(O)NCO$, CH_2Cl_2, $20\,^\circ C$, 1 h; 2) K_2CO_3, MeOH, $20\,^\circ C$, 1 h; j) DIBAL-H, CH_2Cl_2, $-78\,^\circ C$, 1.5 h; k) DMP, CH_2Cl_2, $20\,^\circ C$, 1 h; l) 1) **2**, (+)-Ipc_2BCl, Et_3N, Et_2O, 1.5 h; **5**, $-78 \rightarrow -27\,^\circ C$, 16 h; 2) H_2O_2(30% aq)/MeOH, pH7 buffer, $0\,^\circ C$, 1 h; m) $Me_4NBH(OAc)_3$, MeCN/AcOH, $-25 \rightarrow 20\,^\circ C$, 16 h; n) HF·py, THF, $20\,^\circ C$, 16 h. LiTMP = lithium 2,2,6,6-tetramethylpiperidide, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, TEMPO = 2,2,6,6-tetramethyl-1-piperidinoxyl, BAIB = [bis(acetoxy)iodo]benzene, [18]c-6 = [18]crown-6, KHMDS = potassium bis(trimethylsilyl)amide, DIBAL-H = diisobutylaluminium hydride, Ipc = isopinocampheyl, py = pyridine.

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excellent agreement to those of a natural sample (^{1}H and ^{13}C NMR, IR, TLC, $[\alpha]_D^{20} = +13.0$ ($c = 1.09$, MeOH).

In conclusion, this total synthesis of (+)-discodermolide proceeds in 27 steps and 7.7% overall yield for the longest linear sequence starting from commercial methyl (*S*)-3-hydroxy-2-methylpropionate. The three key subunits were synthesized efficiently using boron-mediated *anti*-selective aldol reactions of chiral ketones (*S*)-**6**, (*S*)-**11**, and (*S*)-**17**. This synthesis has the potential to provide useful quantities of (+)-discodermolide, which will allow detailed biological evaluation, as well as offering a variety of options for analogue chemistry.

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Cofactor-Bound Cross-Linked Enzyme Crystals (CLEC) of Alcohol Dehydrogenase**

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The use of dehydrogenases in organic synthesis is often limited by the intrinsic instability of enzymes and their nicotinamide cofactors.[1] The protein part of the molecule can be efficiently stabilized by several techniques such as directed evolution,[2] immobilization,[3] and protein crystallization and cross-linking.[4] The latter approach has turned out to be especially efficient in producing robust and productive biocatalysts for chemical synthesis.[5] Here, we expand this approach to the stabilization of the cofactor part of the dehydrogenase molecule. Horse liver alcohol dehydrogenase (HLADH) was crystallized in the presence of reduced nicotinamide adenine dinucleotide (NADH), and the resulting crystals were treated with glutaraldehyde to yield the cross-linked enzyme crystals (CLECs). The crystallized and cross-linked HLADH was first introduced by Lee et al., and it demonstrated good activity (26% of that in solution) and an increased stability of the cross-linked crystals in the presence of zinc salts.[6] In this work, we use this system to address two main questions: 1) Is a cofactor more stable when bound inside the enzyme crystal? 2) Is it possible to regenerate a cofactor using a coupled substrate system, thus making HLADH-NADH-CLEC a useful catalyst for organic synthesis?

The activity of soluble enzyme and various HLADH-CLEC preparations was compared in the reduction of 6-methyl-5-hepten-2-one (**1**) in the presence of isopropanol for cofactor regeneration (Scheme 1). The results, presented in Table 1, afford several conclusions. The HLADH-NADH-CLECs exhibit higher activity when HLADH is cocrystallized with a cofactor and an inhibitor, DMSO. In this case, the resulting complex exhibited 64% of the activity of the soluble enzyme in the absence of an exogenous cofactor. DMSO seems to be

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