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Investigation of the Stereoselective Synthesis of the Indane Dimer PH46A, a New Potential Anti-Inflammatory Agent

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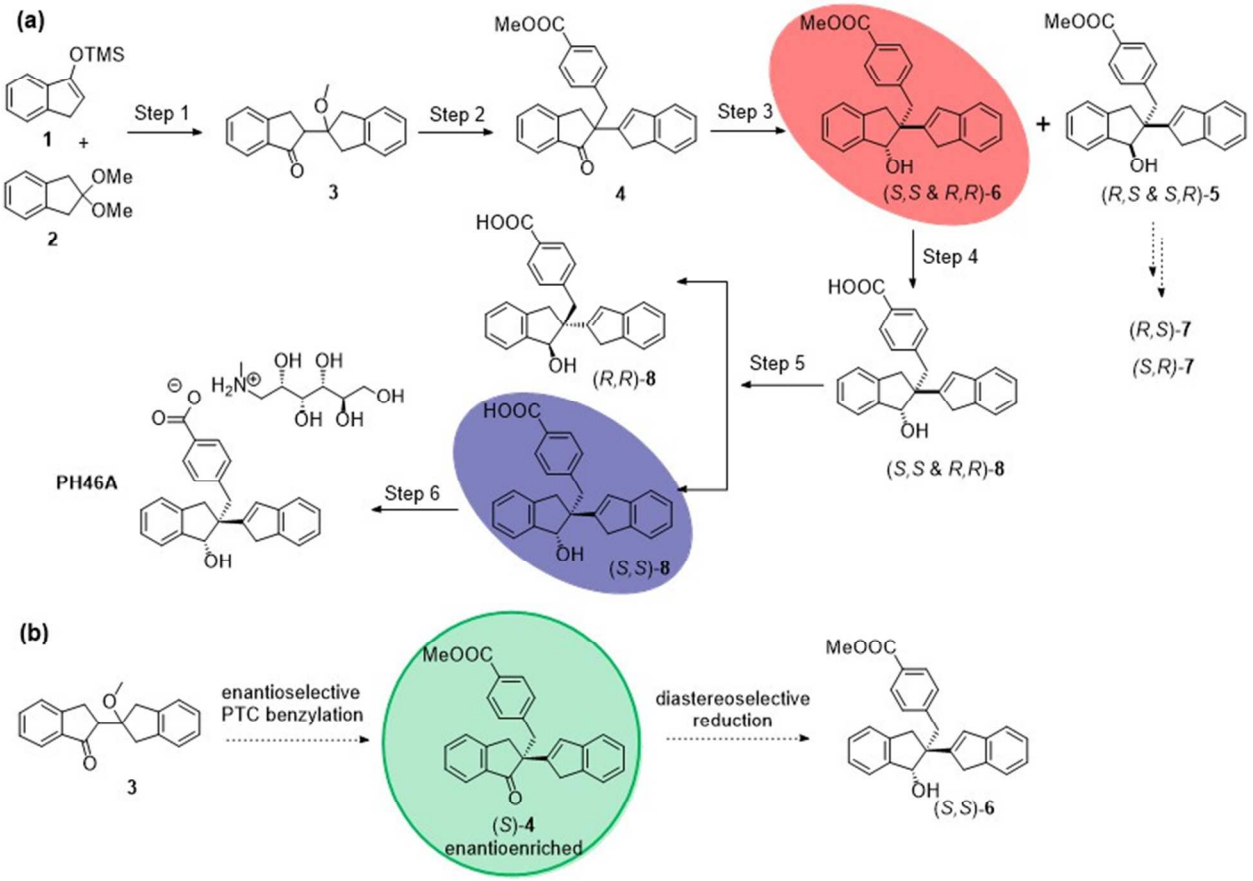
KEYWORDS: PH46A, phase transfer catalysis (PTC), chiral, achiral, indane dimer

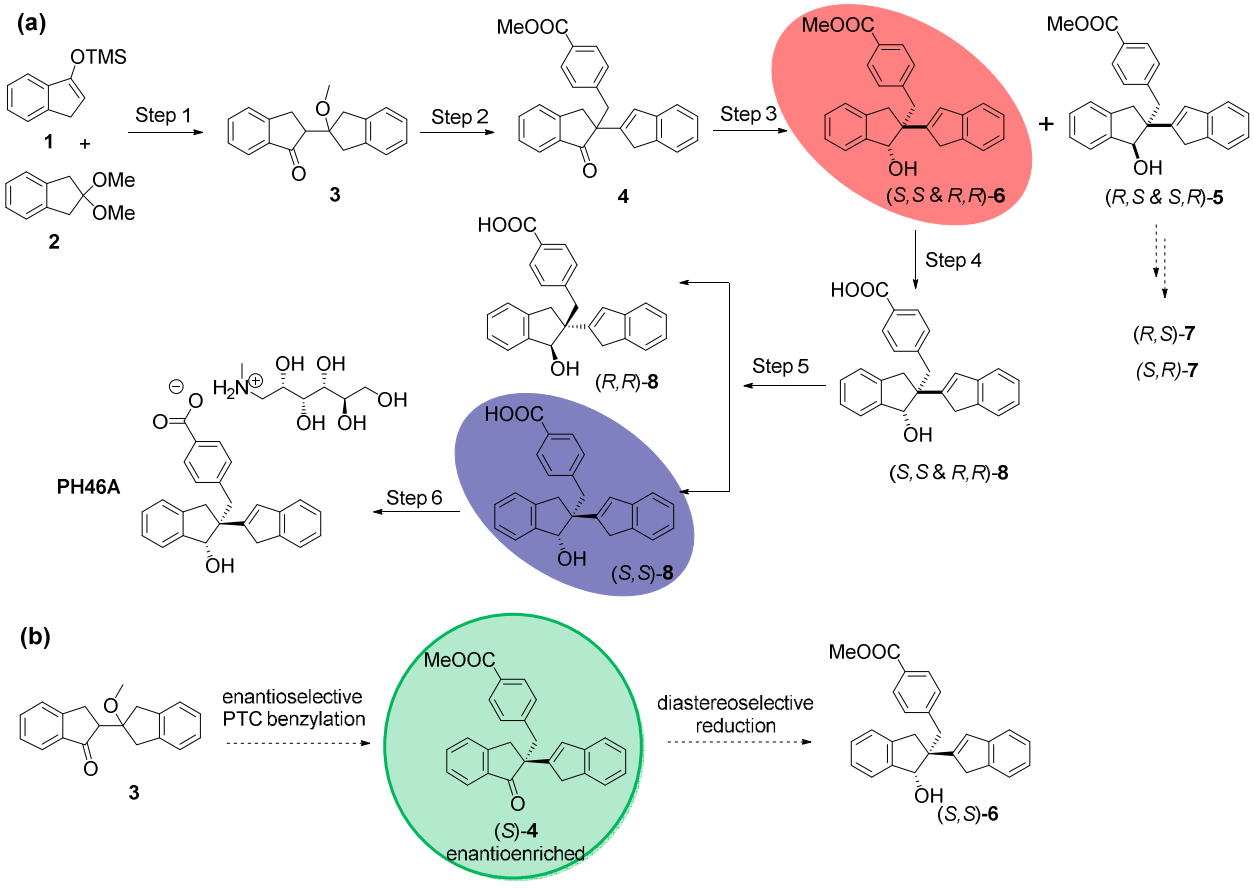

(a)
OTMS
1
+
OMe
OMe
2
Step 1
3
Step 2
MeOOC
4
Step 3
MeOOC
OH
(S,S & R,R)-6
MeOOC
OH
(R,S & S,R)-5
Step 4
HOOC
OH
(S,S & R,R)-8
(R,S)-7
(S,R)-7
Step 5
HOOC
OH
(R,R)-8
HOOC
OH
(S,S)-8
Step 6
PH46A
OH
(b)
3
enantioselective
PTC benzylation
MeOOC
(S)-4
enantioenriched
diastereoselective
reduction
MeOOC
OH
(S,S)-6

ABSTRACT: PH46A, belonging to a class of 1, 2-indane dimers, has been developed by our research group as a potential therapeutic agent for the treatment of inflammatory and autoimmune diseases. The initial synthetic route to PH46A gave a low overall yield, due in large part to the generation of undesired diastereoisomer **5** and the unwanted enantiomer (*R*,*R*)-**8** during the synthesis. The aim of this work was to carry out a comprehensive investigation into the stereoselective syntheses of PH46A. Significant progress was made on the ketone reduction step, where the use of triisobutylaluminum [TiBA, $Al(iBu)_3$] afforded high selectivity for the target diastereoisomer (*rac*)-**6**, compared to the unfavorable ratio obtained using a previous process, and enabled a multi-kilo scale synthesis of PH46A in a GMP environment. Further, a brief proof-of-principle investigation was carried out using an achiral phase transfer catalyst (PTC) for alkylation at the methine carbon of the parent indanone.

INTRODUCTION

The new chiral chemical entity PH46A, 6-(methylamino)hexane-1,2,3,4,5-pentanol 4-(((1*S*,2*S*)-1-hydroxy-2,3-dihydro-1H,1′H-[2,2-biinden]-2-yl)methyl)benzoate, was previously synthesized by our research group[1] and shown to have potential therapeutic activity in the areas of inflammation and autoimmune diseases, including Inflammatory Bowel Disease.[2] PH46A recently completed a First-in-Man Phase I clinical trial study.[3] The original synthetic route to this 'first-in-class' molecule included conventional organic chemistry and preparative chiral HPLC separation (Scheme 1a).[2]

Scheme 1**.** (a). Original Synthetic Route to PH46A with Key Intermediates Generated from Steps 3 and 5; (b). Proposed Stereoselective Synthesis of the Desired Isomer (*S, S*)-**6**.



The overall yield of the original route (Scheme 1a) was less than satisfactory, due to the generation of the undesired diastereoisomer (*rac*)-**5** and the unwanted enantiomer (*R*,*R*)-**8**. The reduction of ketone **3** (step 3) was carried out using sodium borohydride ($NaBH_4$), which afforded a 60:40 ratio of the unwanted diastereoisomer (*rac*)-**5** and desired diastereoisomer (*rac*)-**6**.[2] Bulkier reagents, such as L-Selectride, gave even greater selectivity for the undesired isomer, while conceptually different reductants, such as DIP-chloride and CBS reagent, gave poor conversion.[4] The separation of diastereoisomers **5** and **6** also proved challenging, especially on larger scales, due to their similar chemical and physical properties. Therefore, the development of a scalable stereoselective route for the synthesis of PH46A was desirable to eliminate or reduce the cost burdens of generating the undesired diastereoisomer and of carrying out a large scale separation of the enantiomers. The current article describes our initial work towards this goal, which underpinned the kilo-scale GMP manufacture of PH46A. Further exploration is ongoing to optimize the synthesis and it is anticipated that a combination of enantioselective alkylation to afford ketone **4** as a single enantiomer and subsequent substrate-controlled diastereoselective reduction will ultimately lead to a highly stereoselective synthesis of PH46A (Scheme 1b).

Our initial efforts were directed towards optimization of the diastereoselectivity in step 3 (Scheme 1a), as even moderate improvements could have immediate impact upon yields and purification in planned scale-up work. Prior work had shown $NaBH_4$ in methyl *tert*-butyl ether (MTBE) to be unreactive, probably due to the extremely low solubility of this reagent in simple ethers solvents. $NaBH_4$ in diglyme and $LiBH_4$ in diethyl ether, tetrahydrofuran (THF) and diglyme combinations were briefly explored in this study, without leading to any significant improvements. Meerwein-Ponndorf-Verley-Oppenauer (MPVO) reduction and equilibration

systems were also investigated. Initial trial using aluminium(III) isopropoxide ($Al(OiPr)_3$) gave high selectivity in some cases, however, the high dilution required and low reaction rate left a scalable process out of reach. The use of neodymium(III) isopropoxide ($Nd(OiPr)_3$) along with a simple zeolite catalyst has been reported to promote the racemization of chiral alcohols.[5] When applied to our system, a thermodynamic product distribution was targeted as a result, in contrast to the kinetic product mixture expected from metal borohydride reduction, however, results were disappointing. Finally, a scalable, selective process was developed using triisobutylaluminum, [TiBA, ($Al(iBu)_3$)], a reagent not often encountered as a carbonyl reducing agent.

The second part of this study comprised a brief investigation of achiral phase transfer catalyst (PTC)-promoted benzylation of ketone **3**, to support future development of the enantioselective variant, which had been identified as a particularly interesting avenue of research.[6] Unlike the use of a chiral amide base, such PTC reactions are catalytic in the source of chiral information and typically do not require the exclusion of air or moisture.[7]

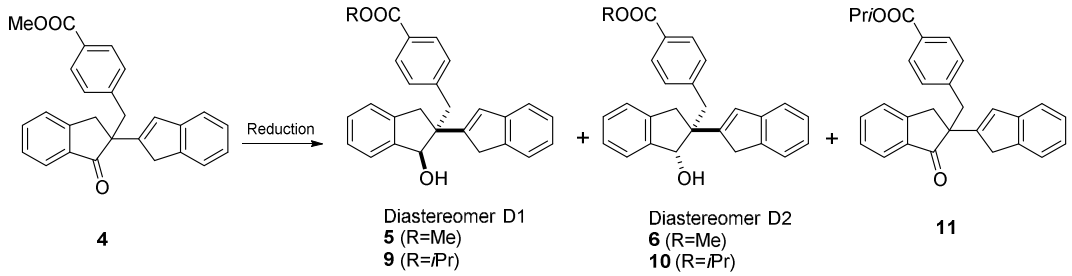
RESULTS & DISCUSSION

The absolute stereochemistry of PH46 (parent compound of PH46A) had previously been established as *S* configuration at both C-1 and C-2.[1] This configuration was shown to be fundamental to the potent anti-inflammatory effects of the molecule.[2] For simplicity and clarity, the descriptors D1 (with non-target configuration, *S,R* & *R,S*) and D2 (with target configuration, *S,S* & *R,R*) are used throughout the discussion for the Me-, isopropyl (*i*Pr)- and *t*-butyl (*t*Bu)-ester derivatives of hydroxyl acids **7** and **8**. Reference standards for all isomers were prepared independently to avoid ambiguity in analyses. Experimental procedures and analytical data are given in the supporting information (Figures S1 and S2).

Diastereoselective reduction

Benchmark reaction using $NaBH_4$ in MeOH A standard reduction of ketone (*rac*)-**4** was first carried out on a 5 g scale, using conditions previously described.[2] The reaction was complete in under 2 h, resulting in the expected 3:2 diastereoisomeric mixture of D1 (**5**) and D2 (**6**) (Scheme 2 & supporting information Figures S3 & S4).

Scheme 2**.** Reduction of Ketone **4**.



Reduction with $NaBH_4$ in isopropanol (IPA) or diglyme As noted in the introduction, previous work in our group had shown that bulky reducing agents increased selectivity towards the unwanted diastereoisomer **5**. We considered that the poor selectivity observed in reduction of ketone **4** using $NaBH_4$/MeOH might be attributable in part to rapid reaction of $NaBH_4$ with MeOH to form various reductants of the general formula $H_nB(OMe)_{(4-n),}$[8] in which one or more of the hydrides present may be reactive, each potentially with differing selectivity as a function of steric and electronic changes. Therefore the effect of a smaller reductant was explored, beginning with unmodified $NaBH_4$ in IPA (Table 1), a solvent which is largely unreactive towards this reducing agent.[9] However, all reactions were extremely slow at RT and at 50°C. In all cases, the initial diastereoisomeric ratio (d.r.) D1:D2 (at RT or 50°C) was approximately 1:1. In addition, an extra set of peaks with longer retention times (reversed phase-HPLC) and with ratios roughly corresponding to those of the starting material and expected products was observed. These were identified as *i*Pr-esters **9**, **10** and **11**; data for these products are given in

the supporting information (Scheme S1, Figures S5 and S6). As the transesterification increased as a function of borohydride stoichiometry, it appears that the process was promoted by a boron-containing species. On extended heating (28 h), some changes to d.r. were observed, with the desired isomer **6** being predominant (D1:D2 up to 30:70) in Entries 1C and 1D. However, this was accompanied by increased levels of byproduct formation (including some hydrolysis to the acids) and it was not possible to determine whether this was a genuine change in selectivity or the result of side-reactions preferentially removing diastereoisomer D1. Interestingly, at least two of the hydrides of $NaBH_4$ seemed to be active, given the conversions in Entries 1A and 1B.

Table 1**.** Reductions of Ketone **4** using $NaBH_4$ in IPA.

Entry	Conditions	D1:D2 ratio (area% conversion)		
		16 h/RT[a]	+4 h/50°C[b]	+28 h/50°C[b]
1A	0.25 eq $NaBH_4$	50:50 (2%)	50:50 (10%)	47:53 (47%)
1B	0.5 eq $NaBH_4$	48:52 (3%)	49:51 (17%)	42:58 (95%)
1C	1.0 eq $NaBH_4$	46:54 (3%)	48:52 (34%)	42:58 (>99%)[c]
1D	2.0 eq $NaBH_4$	44:55 (6%)	48:52 (62%)	31:69 (>99%)[c]

Conditions: IPA (100 mL/g). [a]Ratios measured from Me-ester peaks by HPLC; [b]Ratios measured from *i*Pr-ester peaks by HPLC; [c]Accompanied by significant hydrolysis to **7** & **8** and other byproducts.

Table 2**.** Reductions of Ketone **4** using $NaBH_4$ and $LiBH_4$ in Diglyme.

Entry	Conditions	D1:D2 ratio (area% conversion)		
		3 h/RT[a]	overnight/RT[a]	+8 h/90°C[a,b]
2A	1 eq $NaBH_4$	58:42 (8%)	56:44 (12%)	46:54 (85%)
2B	1 eq $NaBH_4$/TEA	60:40 (5%)	56:44 (6%)	48:52 (>99%)
2C	0.25 eq $LiBH_4$	63:37 (18%)	60:40 (31%)	54:46 (92%)
2D	0.5 eq $LiBH_4$	61:39 (23%)	58:42 (49%)	50:50 (97%)
2E	1.0 eq $LiBH_4$	60:40 (33%)	56:44 (79%)	51:49 (>99%)

Conditions: Diglyme (100 mL/g). [a]Ratios measured from Me-ester peaks by HPLC. [b]Reduced accuracy due to byproduct formation.

Subsequent reactions were carried out in diglyme, chosen as an aprotic solvent in which both $NaBH_4$ and $LiBH_4$ are soluble. A pair of reactions (Table 2, Entries 2A & 2B) were carried out using $NaBH_4$, both with and without TEA (1 eq.), which has been reported[4] to enhance reactivity. The reactions were rather slow at RT and showed similar selectivity (at low conversion) to the

$NaBH_4$/MeOH system. Higher conversation on heating was observed, but was accompanied by some degradation. TEA had no significant effect in our system.

Reductions using $LiBH_4$ $LiBH_4$ is often more versatile than $NaBH_4$ in reductions, as it is soluble in a greater range of aprotic solvents, such as THF, diethyl ether, MTBE and diglyme. After an initial test with a large excess (5 eq.) of $LiBH_4$ in THF at RT overnight demonstrated that the ester was resistant to reduction, a set of reactions in diglyme was carried out (Table 2, Entries 2C-2E) to allow direct comparison with $NaBH_4$. The results showed selectivity similar to that of $NaBH_4$ in diglyme, but higher reactivity. Given time, all the hydrides of $LiBH_4$ reacted, but no useful selectivity was achieved under these conditions.

The Meerwein–Ponndorf–Verley-Oppenauer (MPVO) system The Meerwein-Ponndorf-Verley-Oppenauer reduction is quite different from reductions using borohydride reagents. Firstly, the reaction is, in principle, entirely reversible.[10] Secondly, the steric requirements of the transition state can be quite distinct from those of other hydride donors, as the MPVO system has a 6-membered transition state in which the carbonyl group is coordinated to the metal of the reagent. The reagent, usually $Al(O\textit{i}Pr)_3$[11] or a lanthanide(III) isopropoxide,[12] is theoretically catalytic, but in the case of $Al(O\textit{i}Pr)_3$ larger amounts (>1 eq.) are usually necessary for acceptable rates. Initially, investigations of this system and variants were planned with the aim of obtaining the thermodynamic product rather than the kinetic product as expected from the use of $NaBH_4$ or other irreversible reducing agents. However, while the reaction is reversible in principle, the equilibrium position may not be reached due to other factors and so identification of a thermodynamic product distribution is not trivial.

Table 3**.** Reductions of Ketone **4** using Al(O*i*Pr)$_3$ (Entries 3-6) and MPVO-Scrambling of Mixture **5**/**6** using Nd(O*i*Pr)$_3$ (Entry 7).

Entry	Conditions	D1:D2 ratio (area% conversion)			
		2 h	5.5 h	16 h	22 h
3A	2 eq Al(O*i*Pr)$_3$, IPA (100 mL/g), RT	n.r.	n.r.	n.r.	n.r.
3B	2 eq Al(O*i*Pr)$_3$, IPA (100 mL/g), 75°C	-	-	5:95 (28%)[b]	n.c.
3C	2 eq Al(O*i*Pr)$_3$, IPA (5 eq.), TOL (100 mL/g), 75°C	-	-	6:94 (7%)[a]	n.c.
3D	2 eq Al(O*i*Pr)$_3$, TFA (2 eq.), IPA (5 eq.), TOL (100 mL/g), RT	(<2%)	-	-	n.c.
4A	2 eq Al(O*i*Pr)$_3$, IPA (150 mL/g), 95°C (reflux)	[b]5:95 (21%)	[b]5:95 (47%)	-	[b]5:95 (84%)
4B	4 eq Al(O*i*Pr)$_3$, IPA (150 mL/g), 95°C (reflux)	[b]5:95 (44%)	[b]5:95 (76%)	-	[b]6:94 (90%)
4C	0.2 eq Nd(O*i*Pr)$_3$, IPA (150 mL/g), 95°C (reflux)	[a]20:80 (<2%)	[a]20:80 (2%)	-	[b]23:77(12%)
5	2 eq Al(O*i*Pr)$_3$, IPA (10 mL/g), 110°C (reflux)	[b]12:88 (26%)	[b]13:87 (42%)	-	[b]23:77 (53%)
6A	4 eq Al(O*i*Pr)$_3$, IPA (20 mL/g), 95°C (reflux)	-	-	-	[b]20:80 (75%)
6B	4 eq Al(O*i*Pr)$_3$, IPA (50 mL/g), 95°C (reflux)	-	-	-	[b]11:89 (87%)
6C	4 eq Al(O*i*Pr)$_3$, IPA (200 mL/g), 95°C (reflux)	-	-	-	[b]5:95 (83%)
7	Nd(O*i*Pr)$_3$ (0.2 eq.), acetone (0.5 eq.), TOL, 50°C	89:11	-	-	-

Conditions: see table. [a]Ratio measured from Me ester peaks at low conversion; [b]Ratio measured from *i*Pr-ester peaks at low conversion; TOL=toluene; n.r.=no reaction; n.c.= no change; (-)=not determined. Scale: 1 mmol ketone **4** except for Entry 5 (10 mmol).

Table 4**.** Reductions of Ketone **4** using Al(*i*Bu)$_3$.

Entry	Conditions	D1:D2 ratio[a] (area% conversion)	
		2 h	+72 h
8A	1 eq Al(*i*Bu)$_3$ (2 min addn), TOL (25 mL/g), 5°C 30 min, then RT	8:92 (>99%)	n.a.
8B	1 eq Al(*i*Bu)$_3$ (30 min addn), TOL (25 mL/g), -10 to 0°C 2 h, then RT	6:94 (92%)	n.c.
8C	1 eq Al(*i*Bu)$_3$ (2 min addn), TOL (25 mL/g), -10 to 0°C 2 h, 0.5 eq **4** added, then RT	7:93 (97%)	13:87 (25%)
8D	2 eq Al(*i*Bu)$_3$ (30 min addn), TOL (25 mL/g), -10 to 0°C 2 h, then RT	11:89 (>99%)	n.c.
9A	1 eq Al(*i*Bu)$_3$ (30 min addn), DCM (25 mL/g),	13:87 (>99%)	n.c.
9B	1 eq Al(*i*Bu)$_3$ (30 min addn), THF (25 mL/g),	<1%	n.a.
10A	1 eq Al(*i*Bu)$_3$ (30 min addn), TOL (25 mL/g), -78°C to RT, overnight	6:94 (>99%)	n.a.
10B	1 eq Al(*i*Bu)$_3$ (30 min addn), TOL (12 mL/g), -10 to 0°C, then RT	7:93 (>99%)	n.a.
10C	1 eq Al(*i*Bu)$_3$ (30 min addn), TOL (50 mL/g), -10 to 0°C, then RT	7:93 (>99%)	n.a.
10D	1 eq Al(*i*Bu)$_3$ (60 min addn), TOL (12 mL/g), -10 to 0°C, then RT	7:93 (>99%)	n.a.

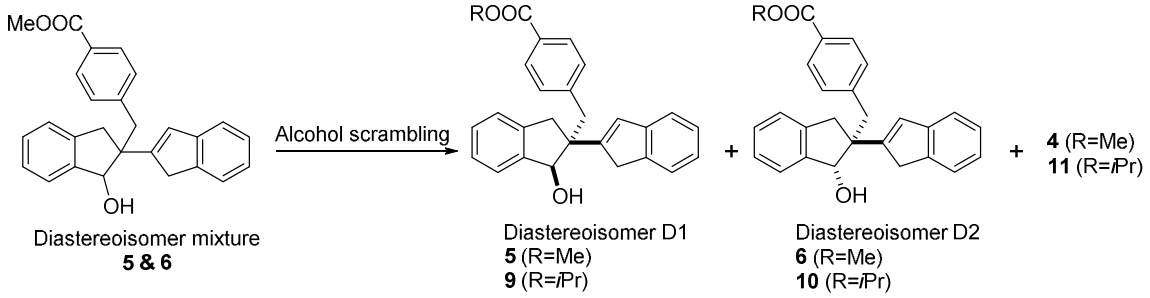
Conditions: see table. [a]Ratio measured from Me ester peaks; TOL=toluene; n.a.=not applicable (reaction quenched at 2 h); n.c.= no change. Scale: 1 mmol ketone **4** except for Entry 10 C (0.5 mmol) and Entry 10D (20 mmol).

MPVO reductions with IPA as both solvent and hydride donor are common, with the equilibrium being driven by the vast excess of the alcohol present.[13] Alternatively, toluene is often used as the main solvent with a smaller excess of IPA. In this case the equilibrium can be driven by loss of acetone to the vapour phase at elevated temperature.[14] Al(O*i*Pr)$_3$-promoted MPVO reductions were first examined at RT and 75°C (Entries 3A-D, Table 3), using bulk-grade IPA as hydride donor. Reactions were carried out below the reflux temperature for either IPA or toluene, under which conditions acetone removal would not have been efficient. As with reactions of $NaBH_4$ in IPA, these were accompanied by a high level of transesterification to the *i*Pr-esters. Although conversions were low, Entries 3B and 3C showed a surprising degree of selectivity for the desired diastereoisomer, with a ratio of approximately 5:95 in favour of D2 (**6** or **10)** in each case. No further change was observed on extended heating. Investigation showed that the IPA used had a water content of >200 μg/mL, which at the high dilutions used could well have resulted in reagent degradation.

Further experiments were carried out (Entries 4A-C, Table 3) using fresh HPLC-grade IPA with a water content <70 μg/mL. As temperature and dilution were increased for this set of reactions, direct comparisons could not be made, but the effect on conversion appeared to be dramatic (compare Entries 4A and 3B). In Entry 4B, where a larger excess of reagent was used, conversion reached 90% HPLC area after overnight heating. Use of substoichiometric Nd(O*i*Pr)$_3$ (Entry 4C) was not effective; stoichiometric use was not considered for cost reasons. Entries 5 and 6A-6C were conducted in an effort to maximise conversion and improve the practicality of the process. A reaction (Entry 5) was carried out at 10 mmol scale with greatly increased concentration (10 mL/g), which was expected to avoid significant hydrolysis of the reagent by water in the solvent. The temperature was also raised (made possible by the higher reflux

temperature of the more concentrated mixture) to favour conversion by driving off the acetone produced. However, conversion after overnight heating was poor and the D1:D2 ratio was significantly worse than that seen on smaller scales under more dilute conditions. Further investigation on smaller scale (Entries 6A-C, Table 3) was conducted to determine whether this was a temperature or concentration effect, with a high loading of reagent used to favour the conversion, as in Entry 4B. The results showed clearly that increasing concentration led to poorer D1:D2 ratios, as well as reduced conversion. We surmised that the increased concentration favoured the reverse reaction as a result of higher acetone concentration, leading to both lower conversion and more opportunity for equilibration of reaction products. Though the hypothesis of a tendency towards an equilibrium position was supported by the slow erosion of D1:D2 ratio with time in reaction Entry 5, it is still possible that the observed effect was simply due to changes to the nature (e.g. polarity and solvation) of the reaction environment.

Scheme 3. MPVO Scrambling of Diastereoisomer Mixture **5**/**6** using a Neodymium(III) Catalyst.



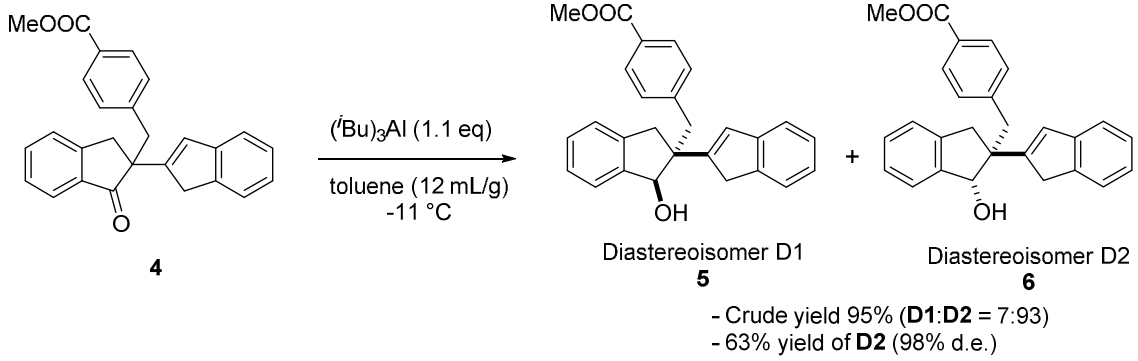
To help understand the MPVO system further, scrambling of a diastereoisomeric mixture of **5** and **6** (d.r. 60:40) using catalytic $Nd(O\textit{i}Pr)_3$ was investigated, with the aim of forming the thermodynamic product by concurrent oxidation and reduction (Scheme 3 and Entry 7 in Table 3). A relatively high loading of acetone (0.5 eq) was used as the redox partner to accelerate the reaction. To our surprise, along with the expected formation of ketone **4**, the undesired isomer **5** prevailed (D1:D2, 89:11) after a relatively short reaction time (2.5 h). While this suggests that

diastereoisomer D1 is the thermodynamic product, from this experiment alone we cannot rule out a selective oxidation of the diastereoisomer D2. To our knowledge, the relevant literature discusses this type of reaction only in terms of racemisation of a single stereocenter or scrambling of a center remote from other stereocentres.[5,12] Our current hypothesis is that the MPVO reduction gives diastereoisomer D2 as the kinetic product when IPA is in vast excess (suppressing equilibration), but the 'scrambling' reaction (with oxidation *via* an Oppenauer-type process) does indeed tend towards a thermodynamic equilibrium with diastereoisomer D1 in excess. However, this has not been unambiguously confirmed.

Reductions using $Al(iBu)_3$ *as reductant* Triisobutylaluminum (commonly abbreviated as TiBA) is most commonly encountered in the recent scientific literature as a polymerization co-catalyst or reagent for aluminum deposition in electronic applications. Nevertheless, it is also an effective reducing agent for aldehydes and ketones. While the neat liquid is pyrophoric, solutions in hexanes and toluene are both readily handled and widely available. Both the mechanism of reduction (a 6-membered cyclic transition state, involving ketone coordination to aluminum) and the steric demands using TiBA are very similar to the MPVO reaction.[11, 15] The key differences are that TiBA reduction is essentially irreversible and that no ketone byproduct is formed, features which we expected would help overcome the low conversions observed with $Al(OiPr)_3$ reductions at practical concentrations. However, the reduction product (the aluminate formed from the alkoxide after ketone reduction) can itself undergo equilibration *via* a MPVO-type mechanism if unreacted ketone is also present.[16] When applied to our system, essentially complete conversion and a promising D1:D2 ratio (8:92) were achieved (Table 4, Entry 8A), also supporting our hypothesis that diastereoisomer D2 is the kinetic product with reductions which proceed through a MPVO-type transition state. Based on the apparent efficiency and clean HPLC

profile of this reaction, this route was explored further (Table 4, Entries 8B-D). Each reaction was examined after 2 h, and after extended stirring at RT to determine whether degradation or equilibration would occur. Overall, the reaction appeared robust. Lower temperature and slow reagent addition resulted in only small improvements to d.e. (Entry 8B). While spiking of extra ketone into the reaction mixture (Entry 8C) led to slight erosion of d.e. (presumably *via* a slow MPVO-type equilibration), mixtures without significant ketone present showed no changes with time. Excess reagent (Entry 8D) lead to no significant side-products, although in this case the D1:D2 ratio (11:89) was slightly less favorable. When dichloromethane (DCM) was used as a solvent (Entry 9A), the diastereoselectivity proved to be slightly poorer. Rather surprisingly, no conversion was observed in THF (Entry 9B); use of a coordinating solvent in this case may have disrupted the coordination between the ketone and the reagent required for reaction. Returning to toluene as solvent (Entries 10A-D), reactions proved largely insensitive to both concentration and temperature; the slight increase in d.r. resulting from a much lower reaction temperature did not warrant the use of cryogenic cooling. Choosing Entry 10B as the most suitable for scaling up, Entry 10D was performed on a 20 mmol scale. The crude HPLC profile and product ratios were consistent as on a small scale and a crude yield of 94% was obtained after work-up.

Scheme 4. Reduction of Ketone **4** using TiBA on 100 g scale.



To test the scalability of the process further, a reaction at 100 g scale was successfully conducted under similar conditions to those in Entry 10B (Scheme 4). The diastereoselectivity of the reaction proved unaffected by scale. Following isolation and an unoptimized trituration purification, the 100 g reaction gave a 63% isolated yield of desired isomer D2 (98% d.e.). Detailed reaction procedure and analytical results are given in the experimental section.

Phase transfer catalyst (PTC)-promoted alkylation of ketone 3

Phase transfer catalysis has long been recognized as a powerful tool in both industrial and academic settings. The oft-quoted advantages include simple experimental operations (typically without exclusion of air or moisture), mild reaction conditions, the low cost of common reagents and catalysts, and the amenability to large scale process chemistry.[17] In our original synthetic route, the quaternary center was introduced in a KO*t*Bu-promoted alkylation in *t*BuOH/diethyl ether, and using methyl(4-bromomethyl)benzoate as electrophile.[2] It was believed that this might readily be substituted by an achiral PTC process, using cheaper and less sensitive reagents, and potentially extended in future to an asymmetric process. In the current study, a short-proof-of-concept investigation was carried out using an achiral PTC. Given the potential instability of the methyl ester functionality in methyl(4-bromomethyl)benzoate under typical phase-transfer conditions, a more robust ester was chosen to simplify testing. *t*-Butyl(4-bromomethyl)benzoate was prepared from 4-(bromomethyl)benzoic acid according to the literature method using *t*-butyl-2,2,2-trichloroacetimidate (TBTA) in a mixture of cyclohexane/DCM/THF at RT.[18] This alkylating agent was used in PTC-promoted alkylations of ketone **3** to afford keto *t*Bu-ester **12** (Figure 1). Standard conditions were chosen, using 25% aq. NaOH:toluene (1:5) and tetrabutylammonium iodide (TBAI) as catalyst. Control reactions, without either ketone **3** or the

alkylating agent, were also carried out. Initially, no PTC was added. HPLC analysis after 1 h of vigorous stirring showed neither significant degradation, nor any uncatalyzed alkylation.

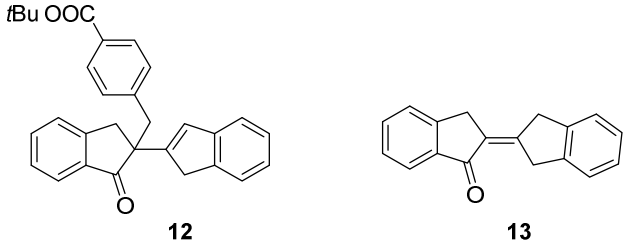


Figure 1. Molecular structures of keto *t*Bu-ester **12** and the unsaturated ketone **13**.

Compound Following addition of the PTC, the HPLC profile for the reaction containing both ketone **3** and alkylating agent showed rapid and clean conversion to the desired alkylation product **12**. The reaction was complete in under 3 h at ambient temperature, while extended stirring had no effect on HPLC profile. NMR spectra and HPLC chromatograms are given in the supporting information (Figures S7-S9). While the control reaction without ketone **3** showed no degradation of the alkylating agent after 16 h, the reaction without alkylating agent resulted in partial degradation of ketone **3**, but the rate of decomposition was sufficiently slow not to be a concern under normal conditions. Among other species, the unsaturated ketone **13** resulting from methanol elimination from **3** was identified in the mixture. Although **13** is a potential intermediate in the desired alkylation process, the species was not observed in PTC- or KOtBu-promoted reactions, indicating that, if formed, it is rapidly consumed in the presence of the electrophile.

CONCLUSION

While investigation of the diastereoselective reduction of keto Me-ester **4** using borohydride reagents failed to improve the medchem route, exploration of Meerwein-Ponndorf-Verley systems was more promising, affording the desired product in good diastereoselectivity. Further

investigations using the related – and yet much less common – TiBA were more successful, leading to a robust, selective and scalable process, and insights into the kinetic *vs* thermodynamic products in our system. This diastereoselective reduction method was successfully employed on multi-kilo scale in a GMP environment, achieving up to 70% yield and >98% d.e. and purity (by area%). Furthermore, the adoption of this step contributed towards a 20% overall cost reduction of the manufacturing of PH46A. The GMP campaign will be described in detail in a separate article.

Progress was also made towards replacing the original KO*t*Bu-promoted alkylation of **3** with a cost-effective, simpler process using phase transfer catalysis. The reaction using a standard achiral quaternary ammonium catalyst proved effective under very simple conditions, though given time constraints this was not scaled-up. Extension to an enantioselective variant is currently under exploration in our laboratory.

EXPERIMENTAL SECTION

All solvents (as anhydrous, HPLC or general process grades) and reagents were purchased commercially and used as received. Air sensitive solutions in septum-sealed bottles were dispensed under a balloon of nitrogen and capped immediately after use. NMR spectra was recorded using a Varian 400 MHz system, and processed using MestreNova software. Chemical shifts are quoted in ppm relative to tetramethylsilane as internal standard. Reverse phase (achiral) HPLC analyses were conducted on Agilent 1200 LC with quaternary pump, column oven and diode array detector with data processing using Chemstation software. Chromatographic conditions: Zorbax C18 XDB column (150 x 4.6 mm) 5 µm; eluent A (water +0.1% TFA *v*/*v*): B (MeOH) 20:80; 1.0 mL/min; column oven 40°C; run time 15 min; injection volume 5 µL; reference standard concentration: 1 mg/mL in diluent. Sample preparation from PTC

alkylations: reaction mixture sample (2.5 μL) was diluted with IPA (1 mL). Sample preparation from reductions: reaction mixture sample (5 μL) was quenched with 3M aq. HCl (100 μL) which was then diluted with IPA (1 mL). For diastereoselective reductions, $Al(OiPr)_3$, $Nd(OiPr)_3$ and $LiBH_4$ were opened and stored in a N_2 glove-bag (no active removal of moisture or oxygen other than gentle N_2 purging) for the duration of the project.

*Representative procedure for MPVO reduction of methyl-ester **4** (Entry 4B):* To a solution of keto Me-ester **4** (98 mg, 0.25 mmol) in IPA (15 mL) was added $Al(OiPr)_3$ (204 mg, 0.5 mmol) in a single portion at RT. The reaction mixture was purged with N_2 and heated to gentle reflux. Conversion was determined by reverse phase HPLC. When reaction was complete, the mixture was acidified using 1M aq. HCl and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated to afford the crude mixture of **9**/**10** as a hard foam.

*MPVO 'scrambling' of diastereoisomeric mixture **5/6** (Entry 7):* A mixture of **5** & **6** (D1:D2, 3:2, 396 mg, 1.0 mmol) was mixed in dry toluene (10 mL) in a flame-dried reaction tube. Toluene was removed under reduced pressure. The procedure was repeated with a second portion of dry toluene (10 mL). The residue was taken up in dry toluene (10 mL), and $Nd(OiPr)_3$ (63 mg, 0.2 mmol) was added. The tube was purged with N_2 and placed in an aluminum heating block (block temperature 55 °C). Dry acetone (74 μL, 1 mmol) was then added, resulting in a change of color from blue to yellow. Conversion was determined by HPLC. The area ratio after 2.5 h at 55 °C was (D1:D2:**11**) 22:3:75. The reaction products were not isolated.

*100 g scale-up reduction of keto Me-ester **4**:* Toluene (1.2 L) and keto Me-ester **4** (99.1 g, 251 mmol) were charged to the vessel, and the mixture was stirred (250 rpm) for 30 min while purging the vessel atmosphere with N_2. The mixture was cooled to an internal temperature of

-12 °C. A solution of Al(*i*Bu)$_3$ (1.1 M in toluene, 219 g, 288 mmol) was pumped into the vessel through PTFE tubing over 1 h. The internal temperature rose and steadied at -11 °C. After addition, the reaction mixture was allowed to warm slowly with stirring for a period of 2 h, reaching an internal temperature of 10 °C. Addition of water (100 mL) resulted in a rise of the internal temperature to 28 °C. Aq. HCl (1 M, 0.6 L) was added, accompanied by a slight further exotherm to 29 °C. The aqueous layer was discarded and the organic phase washed further with aq. HCl (1 M, 0.3 L) and brine (0.3 L). The organics were dried over $MgSO_4$, filtered and concentrated under reduced pressure at 50°C to afford crude **4** as a solid (95 g). The solid was triturated in MTBE (95 mL) for 2 h and left to stand overnight at 5 °C. HPLC analysis of the supernatant at this stage indicated a d.e. of 49%. The solid was collected by filtration, washed with cold MTBE (95 mL in two portions) and dried to afford diastereoisomer **6** (63 g, 63%) with 95% purity and 98% d.e. ^{1}H NMR spectrum is given in Figure 2.

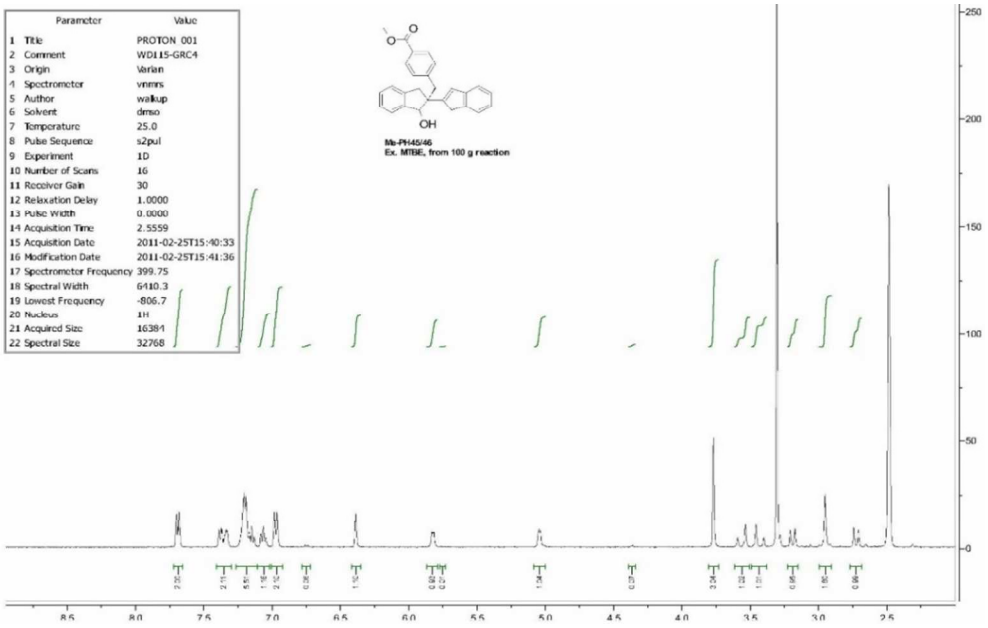


Figure 2. ^{1}H NMR spectrum of hydroxyl Me-ester **6**.

^{1}H NMR (400 MHz, dmso-d_6) δH (ppm): 7.70 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.34-7.40 (m, 2H, Ar-H), 7.14-7.25 (m, 5H, Ar-H), 7.07 (t, *J* = 14.4 Hz, 1H, Ar-H), 6.97 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.39 (s, 1H, CH=C), 5.85 (d, *J* = 7.2Hz, 1H, CHOH), 5.06 (d, *J* = 6.8 Hz, 1H, CHOH), 3.77 (s, 3H, CH_3), 3.56 (d, *J* = 23.2 Hz, 1H, CH_2), 3.42 (d, *J* = 23.2 Hz, 1H, CH_2), 3.20 (d, *J* = 13.6 Hz, 1H, CH_2), 2.96 (s, 2H, CH_2), 2.73 (d, *J*=13.6 Hz, 1H, CH_2).

General procedure for PTC alkylation: Ketone **3** (69 mg, 0.25 mmol) and *t*-butyl(4-bromomethyl)benzoate (68 mg, 0.25 mmol) were taken up in toluene (2.5 mL) and charged to a 20 mm diameter vial. TBAI (5 mg) and 25% aq. NaOH (0.5 mL) were added, and the mixture was stirred at 1500 rpm overnight. The phases were separated and the toluene phase concentrated under reduced pressure to afford the crude product.

ASSOCIATED CONTENT

Supporting Information

Supporting information is available free of charge on the ACS publications website.

The details of experimental procedures for the synthesis of the reference molecules and their corresponding analytical data including NMR spectra and HPLC chromatograms (PDF).

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Frampton, C.-S.; Zhang, T.; Scalabrino, G.; Frankish, N.; Sheridan, H. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun*. **2012**, 68, o323.

(2) Frankish, N.; Sheridan, H. *J. Med. Chem.* **2012**, 55, 5497.

(3) Therapeutics, T.; A study to assess the safety and tolerability of PH46A in healthy volunteers, to measure drug levels in these subjects and to determine the effect of food on the drug's absorption. BioMed Central: ISRCTN Registry, EudraCT: 2013-003717-17, 2014.

(4) Frankish, N.; Sheridan, H. *Unpublished results on Stereochemistry*. **2010**.

(5) Klomp, D.; Djanashvili, K.; Svennum, N.-C.; Chantapariyavat, N.; Wong, C.-S.; Vilela, F.; Maschmeyer, T.; Peters, J.-A.; Hanefeld, U. *Org. Biomol. Chem.* **2005**, 3, 483.

(6) Snyder, S.-E.; Shvets, A.-B.; Pirkle, W.-H. *Helv. Chim. Acta.* **2002**, 85, 3605.

(7) Makosza, M. *Pure Appl. Chem.* **2000**, 72, 1399.

(8) Brown, H.-C. *Organic Synthesis via Boranes*; Wiley-Interscience: New York, 1975.

(9) Brown, H.-C.; Mead, E.-J.; Subba-Rao, B.-C. *J. Am. Chem. Soc.* **1955**, 77, 6209.

(10) Graves, C.-R.; Joseph-Campbell, E.; Nguyen, S.-T. *Tetrahedron: Asymmetry* **2005**, 16, 3460.

(11) Cha, J.-S. *Org. Process Res. Dev.* **2006**, 10, 1032.

(12) Klomp, D.; Maschmeyer, T.; Hanefeld, U.; Peters, J.-A. *Chem. Eur. J.* **2004**, 10, 2088.

(13) Lee, J.; Ryu, T.; Park, S.; Lee, P.-H. *J. Org. Chem.* **2012**, 77, 4821.

(14) Wang, Z. *In Comprehensive Organic Name Reactions and Reagents,* John Wiley & Sons, Inc., 2010.

(15) Winterfeldt, E. *Synthesis* **1975**, 9, 617.

(16) Haubenstock, H.; Davidson, E.-B. *J. Org. Chem.* **1963**, 28, 2772.

(17) Ooi, T.; Maruoka, K. Angew. *Chem. Int. Edit. Engl.* **2007**, 46, 4222.

(18) Halbert, S.-M.; Michaud, E.; Thompson, S.-K.; Veber, D.-F. Protease inhibitors, PCT US Appl. US20020049316, April 25, 2002.