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Bioorganic & Medicinal Chemistry 12 (2004) 6249–6254
Bioorganic & Medicinal Chemistry

Serendipitous discovery of an unexpected rearrangement leads to two new classes of potential protease inhibitors

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Received 13 July 2004; revised 30 August 2004; accepted 30 August 2004
Available online 2 October 2004

Abstract—The pathogenesis of a range of human diseases arises from the aberrant activity of proteolytic enzymes. Agents capable of selectively modulating the activity of these enzymes are of potential therapeutic value. Thus, there is a continuing need for the design of scaffolds that can be used in the development of new classes of protease inhibitors. We describe herein the serendipitous discovery of an unexpected rearrangement that leads to the formation of two novel templates that can be used in the design of protease inhibitors.

1. Introduction

A hallmark of inflammatory disease is the activation and recruitment of various cell types to inflammatory sites. For instance, chronic obstructive pulmonary disease (COPD) is characterized by an influx of macrophages, neutrophils, and T lymphocytes to the lungs.[1] Likewise in asthma, an inflammatory disease of the airways, mast cells, eosinophils, and neutrophils are the major effector cells.[2] The recruitment of these cells is accompanied by the extracellular release of pro-inflammatory mediators, including a range of serine, cysteine, and metallo-proteases.[1,2] Besides their degradative action, the secreted proteases play a prominent role in attenuating the inflammatory response through various actions, including the activation of signaling pathways.[3–5] While many questions remain regarding the precise role that each protease plays in a particular disease state, it is generally believed that selective inhibition of the proteases associated with inflammatory diseases such as COPD (neutrophil elastase, proteinase 3) and asthma (tryptase) may not only lead to new therapeutic agents, but also shed light on the molecular mechanisms underlying the pathophysiology of inflammatory disease.[6,7]

We have recently described the design of a new class of transition state inhibitors of serine and cysteine proteases (Fig. 1, structure (I)) that has the distinct advantage of exploiting favorable binding interactions with both the S and S′ subsites[8] of a target protease.[9,10] The exploitation of multiple binding interactions with the S and S′ subsites is of paramount importance in enhancing potency and, more importantly, attaining maximal selectivity among proteases that have the *same* primary substrate specificity. Thus, inhibitors based on structure (I) could, in principle, exploit the subtle differences that exist in some of the subsites of these proteases.

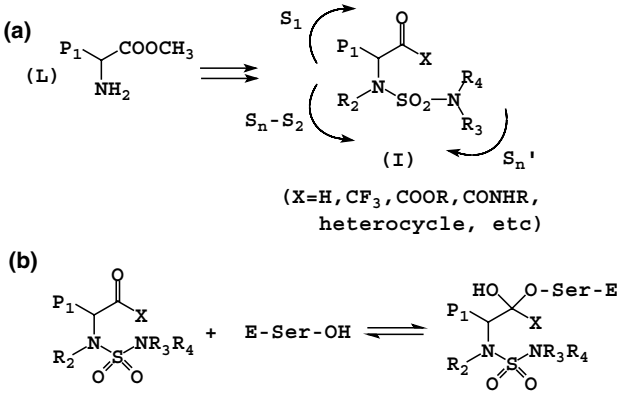

Figure 1. Design and mechanism of action of (I).

Keywords: Protease inhibitors; New scaffolds; Rearrangement.
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doi:10.1016/j.bmc.2004.08.044

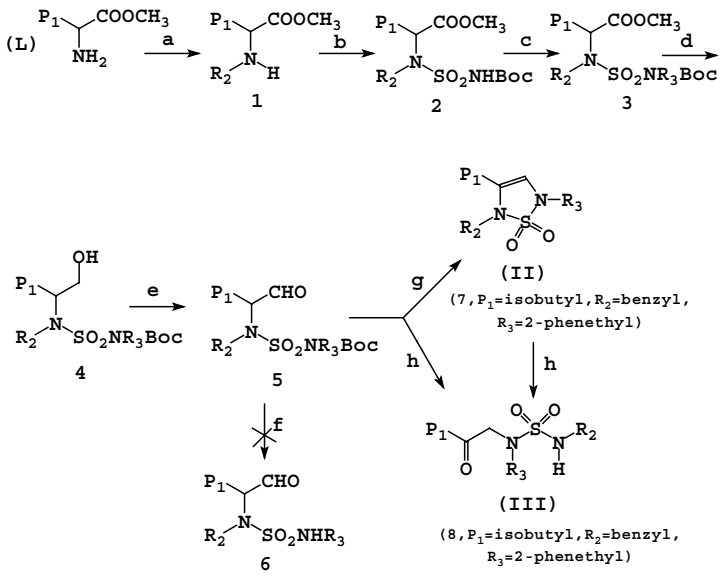

Figure 2. Synthesis of (II) and (III). Reagents: (a) RCHO/$NaBH(OAc)_3$/HOAc; (b) $ClSO_2N{=}C{=}O$/*t*-BuOH/TEA; (c) R_3OH/Ph_3P/DEAD; (d) $LiBH_4$/THF; (e) $(ClCO)_2$/DMSO/TEA; (f) TFA; (g) $AlCl_3/CH_2Cl_2$; (h) *p*-TSA hydrate/CH_2Cl_2/THF/rt.

It was initially envisaged using the general sequence of steps shown in Figure 2 for the synthesis of template (I). During the course of synthetic studies leading to (I), it was noted that aldehyde **5** could be readily transformed into scaffold (II) or (III). We describe herein the results of studies related to this unexpected rearrangement and advance the notion that these observations provide the framework for the design of two new potential classes of protease inhibitors.

2. Chemistry

Compounds **1**–**5** and **7**–**9** were synthesized as shown in Figures 2 and 3.

2.1. Biochemical studies

2.1.1. Screening procedure/human leukocyte elastase (HLE). In a typical inhibition experiment, 10 μL of 7.0 μM HLE was incubated with 10 μL of a 3.50 mM solution of inhibitor in DMSO ([inhibitor]/[enzyme] = 500), 40 μL DMSO and 930 μL 0.1 M HEPES buffer/0.5 M NaCl, pH 7.25 at 25 °C. After 20 min, 10 μL of 70 mM methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide was added and the amount of active enzyme was determined by monitoring the release of *p*-nitroaniline at 410 nm for 2 min. A control (hydrolysis) containing 10 μL of 7.0 μL HLE, 930 μL 0.1 M HEPES buffer, 0.5 M NaCl, pH 7.25, and 50 μL DMSO was run under the same conditions. The remaining activity of HLE was expressed as % remaining activity = $(v/v_0) \times 100$ and is the average of duplicate or triplicate determinations.

2.1.2. Screening procedure/proteinase 3 (PR3). Ten microliters of a 3.40 μM solution of proteinase 3 in 0.1 M phosphate buffer, pH 6.50 were added to a cuvette containing 930 μL 0.1 M HEPES buffer, pH 7.25 containing 0.5 M NaCl, 10 μL DMSO, and 10 μL 1.70 mM inhibitor solution in DMSO (final inhibitor concentration 1.70 μM at an [I]/[E] = 500). After the solution was incubated for 0.5 h at 25 °C, 20 μL of 16 mM 5,5′-dithio-bis(2-nitrobenzoic acid) in DMSO and 20 μL of 6.49 mM Boc-Ala-Ala-Nva-SBzl in DMSO were added and the change in absorbance was monitored at 410 nm for 2 min. A control (hydrolysis) containing 930 μL of 0.1 M HEPES buffer/0.50 M NaCl, pH 7.25, 10 μL PR 3, 20 μL 5,5′-dithio-bis(2-nitrobenzoic acid), 20 μL Boc-Ala-Ala-Nva-SBzl, and 20 μL DMSO was also run under the same conditions. Determinations were performed in duplicate or triplicate and remaining activity was calculated as described for HLE.

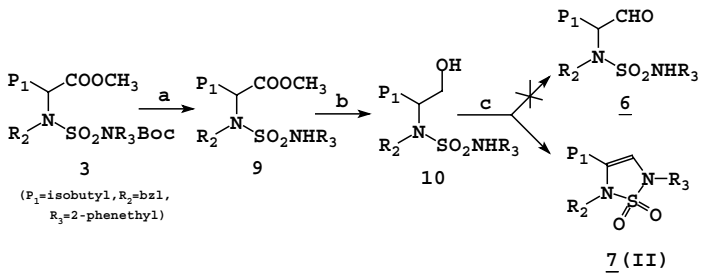

Figure 3. Formation of compound **7**/template (II). Reagents: (a) CF_3COOH/CH_2Cl_2; (b) $LiBH_4$/THF; (c) $(ClCO)_2$/DMSO/TEA.

3. Results and discussion

3.1. Synthesis

We have recently disclosed the design and use of amino acid-derived sulfamide derivatives as potential inhibitors of serine proteases (structure (I), Fig. 1a).[9,10] Structure (I) constitutes a new and versatile class of transition state inhibitors (Fig. 1b) of serine and cysteine proteases capable of exploiting favorable binding interactions with the S and S′ subsites of a target protease. The general synthetic scheme devised for the synthesis of derivatives of (I) with $X = R_4 = H$, envisioned the initial synthesis of key aldehyde **5**, followed by deblocking (Fig. 2). NMR analysis of the crude product formed by treating compound **5** with TFA/CH_2Cl_2 (4:1)/2 h showed the absence of the desired aldehyde **6**. Several other methods for removing the Boc group were then explored, including silica gel/microwave[11] and ammonium cerium(IV)-nitrate/acetonitrile,[12] leading to complex mixtures of products. Surprisingly, treatment of compound **5** with anhydrous $AlCl_3$[13] yielded compound **7** in high yield (structure (II), P_1 = isobutyl, R_2 = bzl, R_3 = 2-phenethyl). Swern oxidation of alcohol **10** also yielded compound **7** in quantitative yield (Fig. 3). To our knowledge, this the first report describing the synthesis of the 2,5-dihydro-[1,2,5]thiadiazole-1,1 dioxide scaffold (II). Thus, this novel template can be readily assembled in two ways from the readily available aldehyde **5** or alcohol **10.**

Treatment of aldehyde **5** with *p*-toluenesulfonic acid monohydrate[14] led to the exclusive formation of **8** (Fig. 2, structure (III), P_1 = isobutyl, R_2 = bzl, R_3 = 2-phenethyl). The structure of compound **8** was confirmed by single crystal X-ray analysis (Fig. 4).

Treatment of compound **7** with *p*-toluenesulfonic acid (*p*-TSA) monohydrate under the same conditions gave compound **8**, suggesting that compound **7** is an intermediate in the reaction leading to the formation of **8** from **5.** A plausible mechanism for the formation of

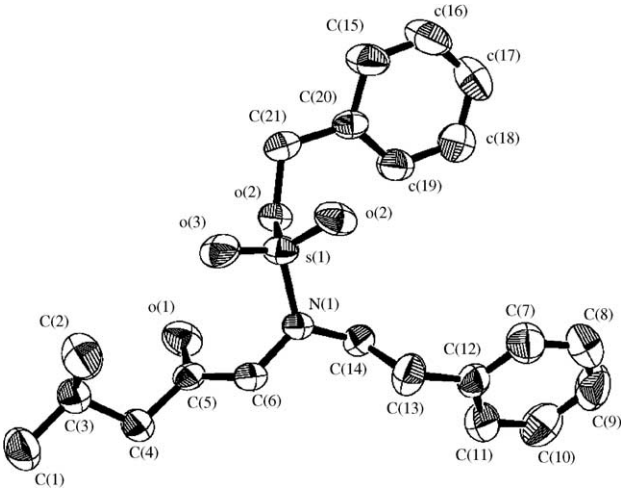

Figure 4. ORTEP drawing of compound **8**.

compounds **7** and **8** is shown in Figure 5 where acid-catalyzed removal of the Boc group is followed by an intramolecular addition and loss of water to give compound **7**/scaffold (II). Acid-catalyzed hydration of **7** followed by ring opening ultimately leads to compound **8**/scaffold (III).

3.2. Biochemistry

The observations cited above, taken together with recent studies related to the design of new scaffolds, suggested that core structures (II) and (III) can be used in the development of protease inhibitors. Specifically, in earlier studies the 1,2,5-thiadiazolidin-3-one-1,1-dioxide template was used in the design of protease inhibitors[15–23] and, more recently, the same template was used as a prototype for the design of protease inhibitors based on surrogate scaffolds such as the 4-imidazolidinone[24] and cyclic sulfamide[25–27] scaffolds. The structure of template (II) suggested that it can be used for the same purpose (Fig. 6a). Indeed, incubation of a 250-fold excess of compound **7** with human leukocyte elastase led

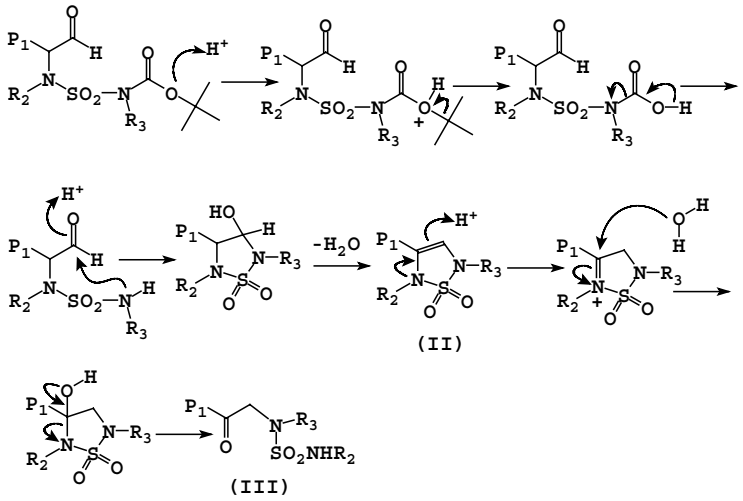

Figure 5. Postulated mechanism for the formation of scaffolds (II) and (III).

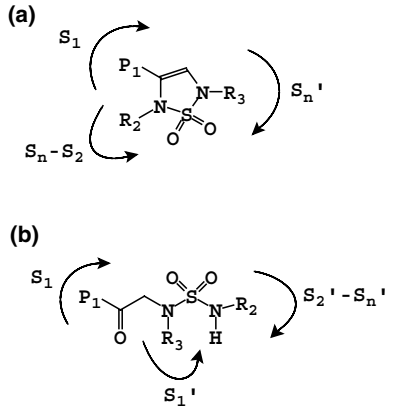

Figure 6. Potential binding interactions of (II) and (III) with the S and S′ subsites.

to weak inhibition (20% inhibition) of the enzyme. Compound **7** was devoid of any inhibitory activity toward human leukocyte proteinase 3. Structure–activity relationship (SAR) studies with template (II) aimed at optimizing potency are currently in progress. Likewise, template (III) is a potential class of transition state inhibitors (Fig. 6b). The results of SAR studies with template (III) will be reported in due course.[28]

4. Experimental

4.1. General

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra of the synthesized compounds were recorded on a Varian XL-300 or XL-400 spectrometers. Human leukocyte elastase and Boc-Ala-Ala-Nva-SBzl were purchased from Elastin Products Co., Owensville, MO. Methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide and 5,5′-dithio-bis(2-nitrobenzoic acid) were purchased from Sigma Chemicals Co., St Louis, MO. Silica gel (230–450 mesh) used for flash chromatography was purchased from Sorbent Technologies (Atlanta, GA). Thin layer chromatography was performed using Analtech silica gel plates. The TLC plates were visualized using iodine vapor and/or UV light. A Hewlett–Packard diode array UV/vis spectrophotometer was used in the enzyme assays and inhibition studies.

4.2. Compound 1

To (DL) leucine methyl ester hydrochloride (2.18 g; 12.0 mmol) and benzaldehyde (1.52 g; 14.3 mmol) in dry 1,2-dichloroethane (30 mL) was added acetic acid (0.96 g; 16.0 mmol), followed by sodium (triacetoxy)borohydride (3.55 g; 16.8 mmol). The reaction mixture was stirred for 3.5 h at room temperature and was then neutralized *dropwise* with 10% aqueous NaOH solution to pH 9–10 with stirring. The organic phase was separated and the aqueous phase was extracted with ethyl ether (3×25 mL). The combined organic phase was dried over anhydrous sodium sulfate. The solvent was removed and the crude product was purified by flash chromatography (ethyl ether/hexane) to give compound **1** (1.87 g, 66% yield). ^{1}H NMR ($CDCl_3$): δ 0.88 (dd, 6H), 1.47 (t, 2H), 1.70–1.85 (m, 2H), 3.30 (t, 1H), 3.61 (d, 1H), 3.71 (s, 3H), 3.82 (d, 1H), 7.19–7.20 (m, 5H).

4.3. Compound 2

A solution of *N*-chlorosulfonyl isocyanate (2.10 g; 14.8 mmol) in dry methylene chloride (25 mL) was cooled in an ice bath. A solution of *t*-butyl alcohol (1.10 g; 14.8 mmol) in dry methylene chloride (10 mL) was added *dropwise* with stirring. After stirring for 15 min at 0 °C, the resulting mixture was added *dropwise* to a solution of compound **1** (3.27 g; 14.8 mmol) and triethylamine (1.50 g; 14.8 mmol) in dry methylene chloride (30 mL) kept in an ice bath. The ice bath was removed and the reaction mixture was stirred for 2.5 h. The reaction mixture was then washed with water (2×25 mL) and the organic phase was separated and dried over anhydrous sodium sulfate. The solvent was removed and the crude product was purified by flash chromatography (ethyl ether/hexane) to give compound **2** (1.87 g; 66% yield). ^{1}H NMR ($CDCl_3$): δ 0.53 (d, 3H), 0.83 (d, 3H), 1.38–1.61 (m, 12H), 3.69 (s, 3H), 4.52 (d, 1H), 4.65 (t, 1H), 4.92 (d, 1H), 7.10–7.52 (m, 5H).

4.4. Compound 3

A solution of compound **2** (41.45 g; 100.0 mmol) in dry THF (260 mL) was treated with triphenyl phosphine (52.99 g; 20.0 mmol), followed by 2-phenyl ethanol (12.20 g; 100.0 mmol) and diethyl azodicarboxylate (31.2 mL; 200.0 mmol). The reaction mixture was stirred for 3 h at room temperature. Removal of the solvent yielded a crude product, which was purified by flash chromatography on silica gel (ethyl ether/hexane) to give compound **3** (45.67 g; 88% yield). ^{1}H NMR ($CDCl_3$): δ 0.61 (d, 3H), 0.89 (d, 3H), 1.40–1.59 (m, 12H), 2.88 (t, 2H), 3.55–3.64 (m, 5H), 4.57–4.68 (m, 2H), 4.91 (d, 1H), 7.18–7.50 (m, 10H).

4.5. Compound 4

To a solution of compound **3** (45.63 g; 87.98 mmol) in dry THF (170 mL) was added *dropwise* a solution of 2 M lithium borohydride in THF (44 mL, 88 mmol), followed by the *dropwise* addition of absolute ethanol (320 mL). The reaction mixture was stirred for 28 h at room temperature and was then cooled in an ice bath and neutralized *dropwise* with 5% aqueous HCl solution to pH 4 with stirring. The solvent was removed to near dryness and water (200 mL) was added. The resulting mixture was extracted with ethyl acetate (3×300 mL) and the organic extracts were combined and dried over anhydrous sodium sulfate. The solvent was removed and the crude product was purified by flash chromatography (ethyl ether/hexane) to give compound **4** (40.12 g; 93% yield). ^{1}H NMR ($CDCl_3$): δ 0.87 (dd, 6H), 1.19 (m, 1H), 1.30 (m, 1H), 1.40 (s, 9H), 1.61 (m, 1H), 2.88 (t, 2H), 3.50–3.70 (m, 4H), 4.07 (m, 1H), 4.46 (dd, 2H), 7.15–7.48 (m, 10H).

4.6. 1-Benzyl-1-[(1-formyl-3-methylbutyl)]-3-phenethyl-3-(carbamic acid *t*-butyl ester)sulfamide 5

A solution of 2 M oxalyl chloride in methylene chloride (2.44 mL; 4.88 mmol) kept in an acetone–dry ice bath was added *dropwise* a solution of dry DMSO (0.51 mL; 3.25 mmol) in dry methylene chloride (4 mL) over a period of 10 min. After the solution was stirred for 0.5 h, the reaction mixture was treated with a solution of compound **4** (1.59 g; 3.25 mmol) in dry methylene chloride (8 mL). Stirring was continued for an additional 0.5 h. A solution of dry triethylamine (1.4 mL; 10 mmol) in methylene chloride (4 mL) was then added, the acetone–dry ice bath was removed, and the reaction mixture was stirred for 15 min. Water (10 mL) was added and the organic phase was separated and washed with 10% aqueous $KHSO_4$ (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL), and then dried over anhydrous sodium sulfate. After the solvent was removed, the resulting crude product was purified by flash chromatography (ethyl ether/hexane) to give compound **5** (1.23 g; 83% yield). ^{1}H NMR ($CDCl_3$): δ 1.88 (dd, 6H), 1.42 (m, 10H), 1.65 (m, 2H), 2.95 (t, 2H), 3.76 (m, 2H), 4.24 (m, 1H), 4.50 (d, 1H), 4.75 (d, 1H), 7.18–7.41 (m, 10H), 9.36 (s, 1H).

4.7. 2-Benzyl-3-isobutyl-5-phenethyl-2,5-dihydro-[1,2.5]thiadiazole-1,1 dioxide 7 (II, P_1 = isobutyl, R_2 = benzyl, R_3 = 2-phenethyl)

(a) A solution of compound **5** (0.61 g; 1.25 mmol) in dry methylene chloride (6 mL) was kept in an ice bath. Anhydrous $AlCl_3$ (0.17 g; 1.25 mmol) was added in one portion, the ice bath was removed, and the reaction mixture was stirred for 3 h at room temperature. The solution was neutralized *dropwise* with 10% aqueous $NaHCO_3$ solution to pH 8–9 while stirring, and the resulting solution was extracted with ethyl acetate (3×60 mL). The organic extracts were washed with brine (2×40 mL) and dried over anhydrous sodium sulfate. Removal of the solvent left a crude product that was purified using flash chromatography. ^{1}H NMR ($CDCl_3$): δ 0.79 (d, 6H), 1.55 (m, 1H), 1.84 (d, 2H), 3.01 (t, 2H), 3.55 (t, 2H), 4.98 (s, 2H), 5.47 (s, 1H), 7.19–7.38 (m, 10H).

(b) To a solution of 2 M oxalyl chloride in methylene chloride (1.92 mL; 3.84 mmol) kept in an acetone–dry ice bath was added *dropwise* a solution of dry DMSO (0.37 mL; 5.12 mmol) in dry methylene chloride (3 mL) over a period of 7 min. After the solution was stirred for 0.5 h, the reaction mixture was treated with a solution of compound **10** (1.00 g; 2.56 mmol) in dry methylene chloride (6 mL) and the reaction mixture was stirred for an additional 0.5 h. A solution of dry triethylamine (1.1 mL; 7.7 mmol) in methylene chloride (3 mL) was added, the acetone–dry ice bath was removed, and the reaction mixture was stirred for 15 min. Water (8 mL) was added and the organic phase was separated and washed with 10% aqueous $KHSO_4$ (8 mL), saturated aqueous $NaHCO_3$ (8 mL), and brine (8 mL). The solution was dried over anhydrous sodium sulfate and the solvent removed to give a crude product, which was purified by flash chromatography (ethyl ether/hexane) to give compound **7** (1.00 g; 100% yield).

4.8. 3-Benzyl-1-[(4-methyl-2-oxo-pentyl)]-1-phenethyl sulfamide 8

(a) Compound **5** (0.72 g; 1.47 mmol) was added in a solution of *p*-toluenesulfonic acid monohydrate (3.80 g; 19.68 mmol) in dry THF (7.5 mL) and dry methylene chloride (4 mL). The reaction mixture was stirred for 40 h at room temperature. The solvent was removed to near dryness and ethyl acetate (50 mL) was added. The resulting solution was washed with saturated aqueous $NaHCO_3$ (3×30 mL), brine (15 mL) and then dried over anhydrous sodium sulfate. The solvent was removed and the crude product was purified by flash chromatography (ethyl ether/hexane) to give compound **8** (0.40 g; 70% yield). ^{1}H NMR ($CDCl_3$): δ 1.89 (d, 6H), 2.04 (m, 1H), 2.16 (d, 2H), 2.83 (t, 2H), 3.42 (t, 2H), 3.95 (s, 2H), 4.24 (d, 2H), 5.10 (t, 1H), 7.12–7.39 (m, 10H).

(b) Compound **7** (0.23 g; 0.62 mmol) was added in a solution of *p*-toluenesulfonic acid monohydrate (1.61 g; 8.30 mmol) in dry THF (3.2 mL) and dry methylene chloride (1.7 mL). The reaction mixture was stirred for 40 h at room temperature. The solvent was removed to near dryness and ethyl acetate (50 mL) was added. The resulting solution was washed with saturated aqueous $NaHCO_3$ (3×30 mL), brine (15 mL) and then dried over anhydrous sodium sulfate. The solvent was removed to give crude compound **8**, which was purified by flash chromatography (0.21 g; 88% yield).

4.9. Compound 9

A solution of compound **3** (1.90 g; 3.66 mmol) in dry methylene chloride (2.5 mL) was treated with trifluoroacetic acid (10 mL). The reaction mixture was stirred for 2 h at room temperature and the solvent was removed on a rotary evaporator to yield a crude product, which was purified by flash chromatography (ethyl ether/hexane) to give compound **9** (1.48 g; 97% yield). ^{1}H NMR ($CDCl_3$): δ 1.62 (d, 3H), 1.82 (d, 3H), 1.43–1.63 (m, 3H), 3.79 (t, 2H), 4.25 (m, 2H), 4.43 (t, 1H), 4.57 (d, 1H), 7.15–7.42 (m, 10H).

4.10. Compound 10

A solution of compound **9** (1.75 g; 4.53 mmol) in dry THF (7 mL) was added *dropwise* a solution of 2 M lithium borohydride in THF (2.3 mL, 4.53 mmol), followed by the *dropwise* addition of absolute ethanol (14 mL). The reaction mixture was stirred for 28 h at room temperature and was then cooled in an ice bath and neutralized *dropwise* with 5% aqueous HCl solution to pH 4 with stirring. The solvent was removed to near dryness and water (15 mL) was added. The resulting mixture was extracted with ethyl acetate (3×30 mL) and the organic phase was combined and dried over anhydrous sodium sulfate. The solvent was removed and the crude product was purified by flash chromatography (ethyl ether/hexane) to give compound **10** (1.57 g; 89% yield). ^{1}H NMR ($CDCl_3$): δ 1.68 (d, 3H), 1.84 (d, 3H), 1.09

(m, 1H), 1.30 (m, 1H), 1.54 (m, 1H), 2.79 (t, 2H), 3.26 (t, 2H), 3.53 (dd, 1H), 3.62 (dd, 1H), 3.93 (m, 1H), 4.26 (d, 1H), 4.40 (d, 1H), 7.15–7.47 (m, 10H).

Acknowledgements

Financial support of this work by the National Institutes of Health (HL57788 to W.C.G. and HL66543 and HL72903 to J.R.H.) and Supergen, Inc. is gratefully acknowledged.

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