Total Synthesis and Protein Kinase Activity of C(7) Methyl Derivatives of K252a

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Received 24 March 1999; revised 12 May 1999

Abstract: Recent efforts in these laboratories have resulted in the development of a synthetic approach to C(7) alkyl analogs in the K252a class of indolocarbazole natural products. Synthesis of 7-*(R)*-methyl K252a (**3a**) and 7-*(S)*-methyl K252a (**3b**) will be described along with their activity against protein tyrosine kinases.

Key words: indolocarbazole, K252a, protein kinase, K252a analogs, synthesis

Protein kinases are vital to the signal transduction pathways which carry external signals to the nucleus of the cell. The disruption of cellular signal transduction via protein kinase (PK) malfunction has been related to the onset of several disease states, including rheumatoid arthritis, systemic lupus erythematosis (SLE), diabetes melitus, Alzheimer's disease, and cancer.[1] While PK inhibition seems a logical target for chemotherapeutic intervention, efforts to prepare PK-specific inhibitors based on ATP have met with limited success due to sequence homology among the many PK isozymes and throughout the large kinase superfamily. However, recent structural data suggests that the homology is limited to the triphosphate binding region, and not the adenosine pocket.[2] Thus, highly specific inhibitors which could take advantage of subtle differences in the nucleoside binding pocket would be invaluable to studies of the cellular roles of individual protein kinases, and in many cases could have significant medicinal value.

Due to their potent inhibition of protein kinase C (PKC), K252a (**1**), and staurosporine (**2**) (Figure 1), two members of the indolocarbazole class of natural products, have attracted great interest throughout the chemical community. The furanosylated congener K252a was isolated independently by Sezaki from *Actinomadura* sp. SF-2370,[3] and by Kase from *Norcardiopsis* sp. K-252.[4] Kase subsequently found K252a to inhibit PKC with an IC_{50} value of 32 nm.

Considerable effort has been focused on the production of numerous analogs of **1**, however, the lack of efficient synthetic approaches to this class of molecules has limited the effort to structures that are readily derived from the natural material. As summarized in Figure 2, the known analogs of K252a have been prepared via single and double Friedel–Crafts alkylation/acylation, oxidation of the lactam methylene, and acylation of the lactam nitrogen. In addition, the literature indicates that derivatization of the carbohydrate moiety has been limited to manipulation of the resident functionality.

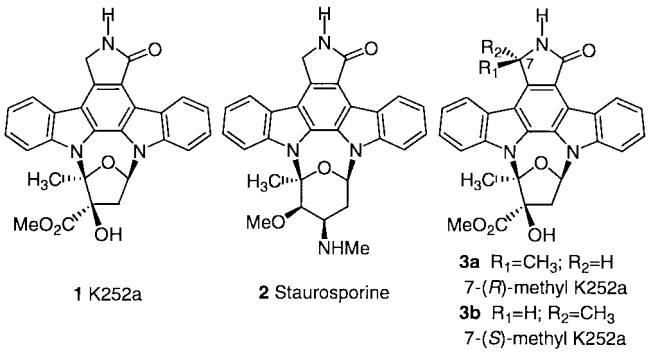


Figure 1 Indolocarbazole natural products and analogs

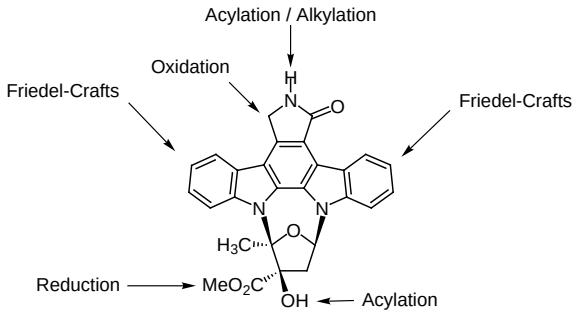
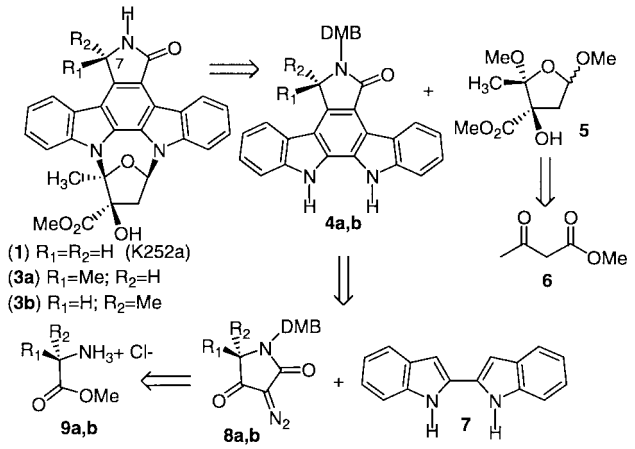


Figure 2 Summary of known K252a analogs

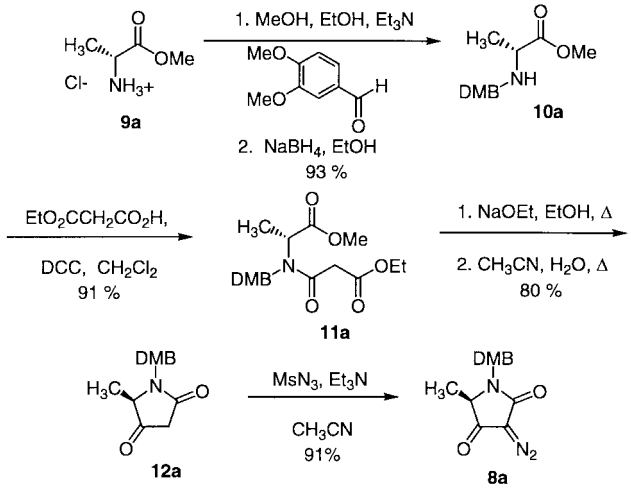
Recently, we devised an efficient synthesis of K252a that enables functionalization of the lactam region and is efficient enough to provide realistic access to a variety of analogs that are not available via derivatization of the natural material.[5] Herein we report extension of this synthesis to the production of C(7) methyl derivatives of K252a (e.g. **3a** and **3b**, Figure 1).

As outlined in retrosynthetic fashion in Scheme 1, we envisioned **3a** as arising via cycloglycosidative coupling of aglycon **4a** with our previously prepared carbohydrate moiety **5**. Aglycon **4a** was expected to derive from the coupling of 2,2'-biindole[6] (**7**) with diazolactam **8a**. As in our K252a synthesis, **5** derives from methyl acetoacetate

(**6**), and the diazolactam originates from a protected α-amino acid derivative.



Scheme 1

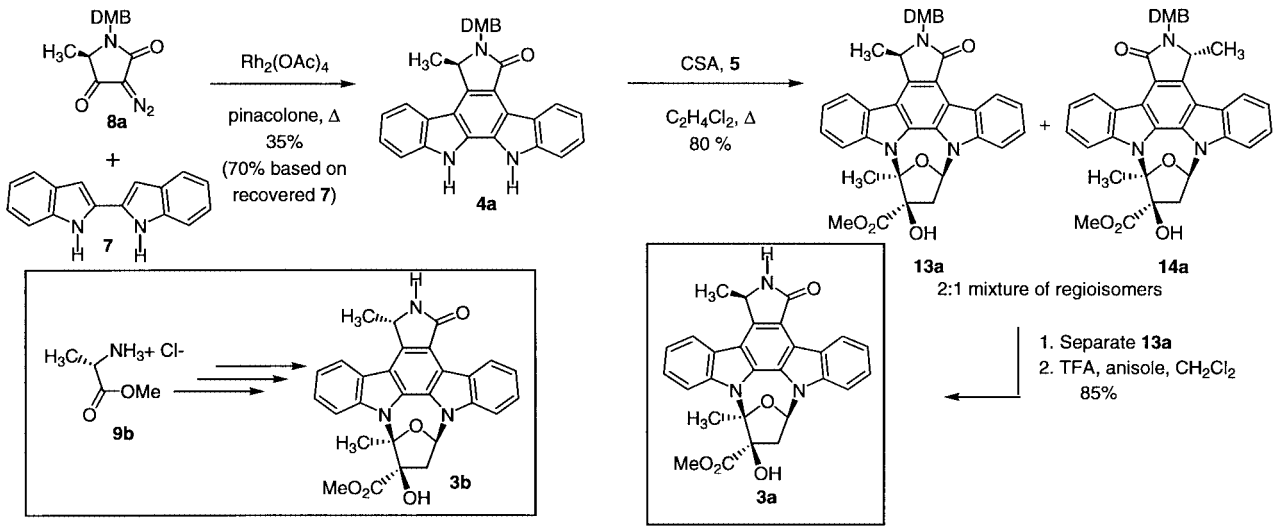


Scheme 2

In the synthetic sense, D-alanine methyl ester hydrochloride (**9a**, Scheme 2) was monoprotected as the dimethoxybenzyl (DMB) derivative via reductive amination with 3,4-dimethoxybenzaldehyde (93% yield).[7] The protected amine **10a** was then coupled to ethyl hydrogen malonate under standard coupling conditions (DCC) to yield the cyclization precursor **11a** in 97% yield. Dieckmann cyclisation[8] of **11a** (NaOEt, EtOH, Δ) furnished the desired lactam which, without further purification, was subjected to decarboethoxylation (CH_3CN, H_2O, Δ) to provide ketone **12** (80% yield, two steps). Diazo transfer from mesyl azide led to the desired diazolactam **8a** in 91% yield (64% overall from D-alanine methyl ester hydrochloride).

In a modification of our previously reported protocol, we found that equimolar amounts **7** and **8a** undergo coupling in the presence of 1 mol% of $Rh_2(OAc)_4$ in pinacolone at reflux (Scheme 3). Under these conditions, aglycon **4a** was produced in 35% yield (70% based on recovered biindole). Recycling of the recovered biindole has allowed the ready preparation of multigram quantities of **4a**.

Cycloglycosidative coupling of **4a** and **5** (CSA, 1,2-dichloroethane, reflux, 72 h) affords an anticipated 2:1 mixture of regioisomers **13a** and **14a** (80% yield), which upon chromatographic separation and deprotection with TFA provides the desired analog **3a** in 85% yield.[9] The diastereomeric analog 7-*(S)*-methyl K252a (**3b**) was similarly prepared using L-alanine methyl ester hydrochloride (**9b**) as the point of departure.



Scheme 3

Table 1 Activity of **1**, **3a**, and **3b** Against Protein Tyrosine Kinases

	IC_{50} (μM)		
	1	**3a**	**3b**
Lck	1.25	5.22	2.70
Fyn	4.59	10.71	6.22
ZAP-70	0.32	>10	4.29
Syk	0.04	3.55	0.19
Itk	0.35	2.02	5.07

To explore the influence of C(7) functionalization we assayed our compounds against a number of protein tyrosine kinases (Table). Although **3a** was found not to be a very potent inhibitor, **3b** showed IC_{50} values ranging from high nM to low mM with the best value being 190 nM against the kinase Syk. Moreover, compound **3b** shows a selectivity profile different from that of the natural product.

In conclusion, the efficient synthesis of K252a developed in our laboratory has been extended to allow stereoselective access to C(7) alkyl derivatives of which 7-*(R)*-methyl K252a (**3a**) and 7-*(S)*-methyl K252a (**3b**) are representative. Importantly these studies have shown that functionalization at the C(7) position does not completely abrogate kinase activity and can in fact induce changes in selectivity. Based on these promising preliminary results, efforts in our laboratories are currently focused on the preparation of a number of additional C(7) alkyl derivatives of K252a.

Unless otherwise stated, reactions were performed in flame-dried glassware under N_2, using freshly distilled solvents. Et_2O and THF were distilled from sodium/benzophenone ketyl. CH_2Cl_2, benzene, and Et_3N were distilled from CaH_2. 1,2-dichloroethane and $BF_3{\cdot}OEt_2$ were purchased from the Aldrich Chemical Co. in Sure/Seal containers and used without further purification. All other commercially obtained reagents were used as received. Unless otherwise stated, all reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using E. Merck silica gel 60 F254 pre-coated plates (0.25 mm). Column or flash chromatography (silica) was performed with the indicated solvents using silica gel (particle size 0.032– 0.063 mm) purchased from Fisher Scientific. In general, the chromatography guidelines reported by Still were followed. Infrared spectra were recorded on a Midac M-1200 FTIR. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-500 spectrometer. Chemical shifts are reported relative to residual solvents $CHCl_3$ (^1H, δ 7.27 ppm, ^{13}C, δ 77.0 ppm), acetone (^1H, δ 2.04 ppm, ^{13}C, δ 29.9 ppm) or DMSO (^1H, δ 2.49 ppm, ^{13}C, δ 39.5 ppm). HRMS were performed at The University of Illinois Mass Spectrometry Center. High performance liquid chromatography (HPLC) was performed on a Waters model 510 system using a Rainin Microsorb 80-199-C5 column, or a Rainin Dynamax SD-200 system with a Rainin Microsorb 80-120-C5 column. Optical rotations were measured on a Perkin-Elmer 241 polarimeter.

Kinase Inhibition Assays

Kinase domains are expressed as Glutathione *S*-transferase (GST) fusion proteins in *Spodoptera frugiperda* (SF9) or High-Five cells, using the Bac-to-Bac expression system (Life Technologies, Paisley, UK). The proteins are then purified to near homogeneity by glutathione-affinity chromatography. The kinase is incubated with [^{33}P]-ATP in a 96-well plate coated with substrate (i.e. poly[Glu, Tyr]4:1). The kinase activity is then measured by a 96-well scintillation counter (i.e. Microbeta, Wallac or Top-Count, Packard). Inhibition by compounds is measured by comparing the relative activity of kinase in the presence and absence of various concentrations of **1**, **3a**, or **3b**. The IC_{50} represents the concentration where 50% of the phosphorylation activity is inhibited.

Amine 10a

To a solution of D-alanine methyl ester hydrochloride (18.1 g, 130 mmol, 1 equiv.), in MeOH (45 mL) was added Et_3N (18.1 mL, 130 mmol, 1 equiv.) followed by addition of 3,4 dimethoxybenzaldehyde (15.1 g, 90.8 mmol, 0.7 eq) as a solution in EtOH (230 mL). The mixture was concentrated under reduced pressure to yield the crude imine as a white solid. The imine was suspended in EtOH (310 mL) and $NaBH_4$ (4.9 g, 130 mmol, 1 equiv.) was added in three to four portions over a 15 minute period. The heterogeneous mixture was stirred for 24 h at r.t. At the end of this period, the EtOH was removed under reduced pressure and the residue was dissolved in 1 M HCl (200 mL). The acid solution was washed with EtOAc (100 mL) and then basified to a pH of 10 using 10 M aq NaOH (10 mL). The basic solution was extracted with CH_2Cl_2 (3 × 100 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated to yield the protected amine **10a** (21.3 g, 93%) as a clear, colorless oil, $[\alpha]^{20}_D$+35.52 (*c* = 1.00, MeOH).

^1H NMR (500 MHz, $CDCl_3$): δ = 1.30 (d, *J* = 7.0 Hz, 3H), 1.81 (br s, 1H), 3.37 (q, *J* = 7.0 Hz, 1H), 3.60 (d, *J* = 12.6 Hz, 1H), 3.72 (m, 4H), 3.84 (s, 3H), 3.87 (s, 3H), 6.78–6.88 (comp m, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.5, 51.0, 51.1, 55.1, 55.1, 55.2, 110.5, 110.9, 119.7, 131.8, 147.5, 148.4, 175.5.

IR (film): ν = 3329 (br w), 2944 (m), 1735 (s), 1513 (s), 1457 (m), 1262 (s), 1199 (s), 1031 (s), 980 (w), 855 cm^{-1}.

HRMS (EI): calcd for $C_{13}H_{19}NO_4$ 253.1314; found 253.1315.

Amide 11a

A flame-dried 500 mL three-necked round bottom flask equipped with an addition funnel was charged with the amine **10a** (25 g, 99 mmol, 1 equiv.), ethyl hydrogen malonate (13.1 g, 99 mmol, 1 equiv.), and CH_2Cl_2 (400 mL). The flask was cooled to 0 °C and a solution of 1,3-dicyclohexyl carbodiimide (20.4 g, 99 mmol, 1 equiv.) in CH_2Cl_2 (160 mL) was added dropwise through the addition funnel over a 15 min period. The ice bath was removed and the mixture was stirred for an additional 3 h at r.t. The mixture was filtered to remove the urea byproduct and the filtrate was washed with H_2O (500 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated to yield amide **11a** as a clear, light yellow oil (34 g, 94%), $[\alpha]^{20}_D$+39.7 (*c* = 0.65, MeOH).

^1H NMR (500 MHz, $CDCl_3$): δ = 1.25 (t, *J* = 7.1 Hz, 3H), 1.40 (d, *J* = 7.2 Hz, 3H), 3.37–3.57 (comp m, 2H), 3.68 (s, 3H), 3.87 (s, 3H), 3.88 (s, 3H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.38–4.74 (comp m, 3H), 6.76–6.87 (comp m, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.0, 14.5, 41.4, 50.6, 52.2, 54.4, 55.8, 61.4, 109.5, 110.9, 111.2, 118.6, 119.6, 128.6, 149.3, 167.0, 167.3, 171.6.

IR (film): ν = 2948 (m), 1741 (s), 1654 (s), 1516 (s), 1261 (s), 1149 (s), 1029 (s), 807 (w) cm^{-1}.

HRMS (EI): calcd for $C_{18}H_{25}NO_7$ 367.1631; found 367.1632.

Lactam 12a

A three-necked round bottom flask was charged with EtOH (18 mL). Na metal (293 mg, 12.8 mmol, 0.94 equiv.) was then carefully added in one portion. When the sodium had completely reacted, ester **11a** (5 g, 13.5 mmol, 1 equiv.) was added dropwise to the mixture as a solution in EtOH (17 mL). The mixture was brought to reflux for 5 min and then allowed to cool to r.t. The EtOH was removed under reduced pressure and the residue was dissolved in H_2O (100 mL). The aqueous layer was washed with EtOAc (50 mL), and acidified to a pH of 2, with 2M HCl (10 mL). The acidic solution was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to yield a yellow oil (3.8 g, 80%). A suspension of this oil in CH_3CN (1130 mL) and H_2O (1 mL) was warmed to reflux open to the air for 3 h. The mixture was cooled to r.t. and the CH_3CN/H_2O mixture was removed at reduced pressure to yield lactam **12a** as a dark yellow oil (2.84 g, 100%) which solidified upon standing, $[\alpha]^{20}_D$+29.1 (*c* = 0.25, MeOH).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.31 (d, *J* = 6.9 Hz, 3H), 3.09 (s, 2H), 3.78 (q, *J* = 6.9 Hz, 1H), 3.85 (s, 3H), 3.86 (s, 3H), 3.97 (d, *J* = 14.7 Hz, 1H), 5.17 (d, *J* = 14.7 Hz, 1H), 6.80 (m, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 15.1, 40.5, 43.6, 55.9, 56.0, 61.7, 111.1, 111.4, 120.7, 127.8, 148.9, 149.4, 168.3, 206.7.

IR (film): ν = 2940 (br m), 1766 (m), 1689 (s), 1603 (w), 1512 (m), 1424 (m), 1252 (s), 1147 (m), 916 (w) cm^{-1}.

HRMS (EI): calcd for $C_{14}H_{17}NO_4$ 263.1158; found 263.1160.

Diazo Lactam 8a

A round-bottom flask was charged with lactam **12a** (1.5 g, 5.7 mmol, 1 equiv.), MsN_3 (763 mg, 6.3 mmol, 1.1 equiv.), and CH_3CN (40 mL). The solution was cooled to 0 °C and Et_3N (0.95 mL, 6.8 mmol, 1.2 eq) was added dropwise to the mixture. After gradually warming to r.t., the mixture was stirred for an additional 3 h and the volatiles were removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL), and washed with a 1M aq NaOH (20 mL). The organic layer was separated, concentrated, dissolved in EtOAc (50 mL), and filtered through a plug of silica gel to yield diazolactam **8a** as a bright yellow foam (1.5 g, 91%), $[\alpha]^{20}_D$+94 (*c* = 0.8, MeOH).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.37 (d, *J* = 6.9 Hz, 3H), 3.76 (q, *J* = 6.9, 1H), 3.88 (s, 6H), 3.85 (s, 3H), 4.04 (d, *J* = 14.9 Hz, 1H), 5.09 (d, *J* = 14.9 Hz, 1H), 6.79–6.83 (comp m, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 15.3, 44.2, 55.9, 55.9, 59.6, 111.1, 111.2, 120.6, 128.0, 148.9, 149.4, 161.3, 189.5.

IR (film): ν = 2928 (br m), 2857 (w), 2125 (s), 1684 (s), 1514 (m), 1401 (m), 1356 (s), 1259 (m), 1025 (w) cm^{-1}.

HRMS (EI): calcd for $C_{14}H_{15}N_3O_4$ 289.1065; found 263.1063.

Aglycon 4a

To a flame-dried three-necked round bottom flask equipped with a condenser were added the diazolactam **8a** (800 mg, 2.8 mmol, 1 equiv.), 2,2'-biindole (650 mg, 2.8 mmol, 1 equiv.), $Rh_2(OAc)_4$ (12 mg, 0.028 mmol, 0.01 eq) and pinacolone (28 mL). The whole was degassed by bubbling a stream of N_2 gas through the solution for 2 h. The mixture was then refluxed for 8 h. The mixture was concentrated to a dark brown foamy solid, which was adsorbed onto silica gel and subjected to flash chromatography (50% EtOAc/hexanes eluent). Compound **4a** was isolated as a pale yellow solid (430 mg, 35%), $[\alpha]^{20}_D$+31.5 (*c* = 0.45, MeOH).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.75 (d, *J* = 6.5 Hz, 3H), 3.73 (s, 3H), 3.74 (s, 3H), 4.46 (d, *J* = 15.2 Hz, 1H), 5.15 (m, 1H), 5.36 (d, *J* = 15.2 Hz, 1H), 6.88 (m, 1H), 6.99 (m, 1H), 7.07 (m, 1H), 7.25 (t, *J* = 7.5 Hz, 2H), 7.41 (m, 2H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.64 (d, *J* = 8.3 Hz, 1H), 8.06 (d, *J* = 7.9 Hz, 1H), 9.53 (d, *J* = 8.0 Hz, 1H), 10.70 (s, 1H), 10.92 (s, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.9, 44.0, 56.1, 56.2, 111.9, 112.6, 112.9, 112.9, 115.2, 117.6, 119.5, 120.2, 121.0, 121.1, 122.8, 123.4, 124.5, 126.0, 126.3, 126.8, 126.9, 127.0, 129.3, 132.2, 137.9, 140.9, 140.9, 149.8, 150.6, 170.0.

IR (film): ν = 3323 (br w), 2931 (w), 1649 (s), 1514 (s), 1320 (s), 1236 (s), 1231 (s), 1140 (m), 1024 (m) cm^{-1}.

HRMS (EI): calcd for $C_{30}H_{25}N_3O_3$ 475.1896; found 475.1896.

Indolocarbazoles 13a and 14a

To a refluxing solution of aglycon **4**a (700 mg, 1.47 mmol, 1 equiv.) and camphorsulfonic acid (34 mg, 0.147 mmol, 0.1 equiv.) in 1,2-dichloroethane (50 mL) was added, via addition funnel over 24h, a solution of furanose **5** (638 mg, 2.9 mmol, 2 equiv.) in 1,2 dichloroethane (32 mL). Reflux was then continued for an additional 48h. The crude mixture was washed with a 10% aq $NaHCO_3$ (20 mL). The aqueous layer was washed with CH_2Cl_2 (3 × 20 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was taken up in a minimum of CH_2Cl_2 and purified by flash chromatography (50% EtOAc/hexanes eluant) to provide a 2:1 mixture of regioisomers **13a** and **14a** (80% yield). Separation of the regioisomers could be achieved using HPLC (190:10:1 CH_2Cl_2:EtOAc:MeOH eluent).

13a, $[\alpha]^{20}_D$+59 (*c* = 0.8, MeOH).

^{1}H NMR (500 MHz, acetone-d_6): δ = 1.78 (d, *J* = 6.5 Hz, 3H), 2.23 (dd, *J* = 5.0, 14.1 Hz, 1H), 2.24 (s, 3H), 3.50 (dd, *J* = 7.4, 14.1 Hz, 1H), 3.76 (s, 3H), 3.77 (s, 3H), 4.01 (s, 3H), 4.45 (d, *J* = 15.2 Hz, 1H), 5.19 (q, *J* = 6.5 Hz, 1H), 5.37 (d, *J* = 15.2 Hz, 1H), 6.92 (m, 1H), 6.99 (m, 1H), 7.08 (m, 1H), 7.16 (dd, *J* = 4.9, 7.4 Hz, 1H), 7.28–7.33 (comp m, 2H), 7.43 (ddd, *J* = 1.1, 7.2, 8.3 Hz, 1H), 7.51 (ddd, *J* = 1.1, 7.2, 8.2 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 8.06 (d, *J* = 7.9 Hz, 1H), 9.50 (d, *J* = 7.8 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.9, 23.5, 43.3, 43.9, 53.5, 56.1, 56.1, 56.1, 85.9, 86.4, 100.5, 109.2, 113.0, 115.5, 115.8, 117.6, 120.1, 120.5, 121.1, 121.2, 122.9, 124.2, 124.8, 125.5, 125.7, 126.5, 127.3, 127.4, 129.8, 132.0, 137.7, 138.5, 141.5, 149.8, 150.7, 169.2, 174.0.

IR (film): ν = 3479 (br w), 3361 (br w), 2949 (w), 1728 (s), 1660 (s), 1583 (m), 1516 (m), 1454 (m), 1257 (m), 1140 (m), 1034 (s), 745 (s) cm^{-1}.

HRMS (EI): calcd for $C_{37}H_{33}N_3O_7$ 631.2319; found 631.2314.

14a, $[\alpha]^{20}_D$+62.7 (*c* = 0.26, MeOH).

^{1}H NMR (500 MHz, acetone-d_6): δ = 1.78 (d, *J* = 6.5 Hz, 3H), 2.22 (s, 3H), 2.27 (ddd, *J* = 1.9, 5.0, 14.0 Hz, 1H), 3.50 (dd, *J* = 7.5, 14.0 Hz, 1H), 3.75 (s, 3H), 3.77 (s, 3H), 4.01 (s, 3H), 4.45 (d, *J* = 15.2 Hz, 1H), 5.15 (q, *J* = 6.5 Hz, 1H), 5.23 (s, 1H), 5.38 (d, *J* = 15.2 Hz, 1H), 6.91 (m, 1H), 6.99 (m, 1H), 7.08 (m, 1H), 7.15 (dd, *J* = 4.9, 7.4 Hz, 1H), 7.31 (q, *J* = 7.7 Hz, 1H), 7.44 (ddd, *J* = 1.4, 7.1, 8.5 Hz, 1H), 7.51 (ddd, *J* = 1.0, 7.2, 8.2 Hz, 1H), 7.87 (d, *J* = 8.3 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 8.11 (d, *J* = 7.9 Hz, 1H), 9.75 (d, *J* = 8.1 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.7, 23.3, 43.1, 43.7, 53.3, 55.4, 56.0, 56.0, 86.0, 86.4, 100.1, 110.0, 112.7, 114.8, 115.4, 118.1, 119.6, 120.4, 120.9, 121.2, 123.1, 123.2, 125.9, 126.0, 126.1, 127.1, 127.2, 127.5, 127.7, 131.9, 138.0, 138.4, 141.1, 149.6, 150.5, 169.4, 173.7.

IR (film): ν = 3479 (w), 3365 (br w), 3057 (w), 2933 (w), 2835 (w), 1738 (s), 1676 (s), 1588 (m), 1511 (m), 1454 (m), 1243 (m), 1140 (m), 1021 (m), 743 (m) cm^{-1}.

HRMS (EI): calcd for $C_{37}H_{33}N_3O_7$ 631.2319; found 631.2314.

7-*(R)*-Methyl K252a 3a

A solution of protected amide **13a** (60 mg, 0.095 mmol, 1 equiv.) and anisole (1.03 g, 9.5 mmol, 100 eq.) in CH_2Cl_2 (2 mL) was treated dropwise with 2,2,2-trifluoroacetic acid (2 mL). After stirring at r.t. for 12 h, the reaction was quenched with a 20% aq $NaHCO_3$ (2 mL). The aqueous layers was washed with CH_2Cl_2 (3 × 5 mL) and the combined organic layers were dried (Na_2SO_4), filtered and adsorbed onto SiO_2. Flash chromatography (50% EtOAc/hexanes eluent) provided **3a** as a pale yellow solid (45 mg, 85%), $[\alpha]^{20}_D$+41 (*c* = 0.1, MeOH).

^{1}H NMR (500 MHz, acetone-d_6): δ = 1.76 (d, *J* = 6.5 Hz, 3H), 2.21 (s, 3H), 2.34 (dd, *J* = 5.0, 14.1 Hz, 1H), 3.52 (dd, *J* = 7.5, 14.1 Hz, 1H), 4.02 (s, 3H), 5.39 (s, 1H), 5.45 (q, *J* = 6.4 Hz, 1H), 7.15 (dd, *J* = 5.0, 7.4 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 1H), 7.44–7.51 (comp m, 2H), 7.65 (s, 1H), 7.79 (d, *J* = 8.3 Hz, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 8.21 (d, *J* = 7.9 Hz, 1H), 9.41 (d, *J* = 7.8 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 21.4, 23.5, 43.3, 53.2, 53.3, 53.5, 85.9, 86.4, 100.5, 109.1, 115.8, 117.7, 120.4, 121.2, 122.9, 124.2, 125.1, 125.4, 125.6, 126.4, 127.4, 127.5, 129.9, 138.4, 139.7, 141.5, 171.6, 174.0.

IR (film): ν = 3407 (br w), 2963 (w), 2928 (m), 2861 (w), 1738 (m), 1678 (s), 1588 (m), 1449 (s), 1392 (m), 1201 (m), 1084 (w), 877 (w), 743 (m) cm^{-1}.

HRMS (EI): calcd for $C_{28}H_{23}N_3O_5$ 481.1638; found 481.1633.

Compounds from the opposite epimeric series (i.e. **13b**, **14b** and **3b**) can also be produced using the same experimental procedures described above and provide the following data:

13b, $[\alpha]^{20}_D$ –14.4 (*c* = 0.23, MeOH).

^{1}H NMR (500 MHz, acetone-d_6): δ = 1.78 (d, *J* = 6.5 Hz, 3H), 2.23 (dd, *J* = 5.0, 14.1 Hz, 1H), 2.23 (s, 3H), 3.50 (dd, *J* = 7.4, 14.1 Hz, 1H), 3.76 (s, 3H), 3.77 (s, 3H), 4.01 (s, 3H), 4.45 (d, *J* = 15.2 Hz, 1H), 5.19 (q, *J* = 6.5 Hz, 1H), 5.37 (d, *J* = 15.2 Hz, 1H), 6.92 (m, 1H), 6.99 (m, 1H), 7.08 (m, 1H), 7.16 (dd, *J* = 4.9, 7.4 Hz, 1H), 7.28–7.33 (comp m, 2H), 7.43 (ddd, *J* = 1.1, 7.2, 8.3 Hz, 1H), 7.51 (ddd, *J* = 1.1, 7.2, 8.2 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 8.06 (d, *J* = 7.9 Hz, 1H), 9.50 (d, *J* = 7.8 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.6, 23.2, 43.1, 43.2, 43.6, 53.2, 55.9, 85.7, 86.0, 86.2, 100.0, 109.1, 112.6, 115.2, 115.8, 117.3, 119.7, 120.3, 120.8, 121.2, 122.5, 124.0, 124.9, 125.3, 125.6, 126.3, 127.1, 127.2, 129.8, 131.8, 137.4, 138.2, 141.2, 149.5, 150.4, 169.1, 173.7.

IR (film): ν = 3473 (br w), 3003 (w), 2950 (w), 1733 (s), 1670 (s), 1586 (m), 1514 (m), 1455 (s), 1259 (s), 1201 (m), 1138 (m), 1026 (m), 747 (s) cm^{-1}.

HRMS (EI): calcd for $C_{37}H_{33}N_3O_7$ 631.2319; found 631.2314.

14b, $[\alpha]^{20}_D$ –15.9 (*c* = 0.18, MeOH).

^{1}H NMR (500 MHz, acetone-d_6): δ = 1.75 (d, *J* = 6.5 Hz, 3H), 2.20 (s, 3H), 2.40 (dd, *J* = 4.9, 14.1 Hz, 1H), 3.55 (dd, *J* = 7.5, 14.1 Hz, 1H), 3.76 (s, 3H), 3.78 (s, 3H), 4.01 (s, 3H), 4.46 (d, *J* = 15.2 Hz, 1H), 5.19 (q, *J* = 6.5 Hz, 1H), 5.30 (s, 1H), 5.36 (d, *J* = 15.2 Hz, 1H), 6.93 (m, 1H), 7.01 (m, 1H), 7.09 (m, 1H), 7.15 (dd, *J* = 4.9, 7.4 Hz, 1H), 7.31 (m, 2H), 7.44 (ddd, *J* = 1.4, 7.0, 8.5 Hz, 1H), 7.52 (ddd, *J* = 1.0, 7.3, 8.2 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 8.13 (d, *J* = 7.9 Hz, 1H), 9.75 (d, *J* = 7.7 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.8, 23.5, 43.5, 43.6, 43.9, 53.4, 55.7, 56.2, 85.8, 86.4, 100.1, 110.0, 113.0, 115.0, 118.1, 119.6, 120.6, 121.1, 121.3, 123.4, 125.9, 126.1, 126.1, 126.2, 127.1, 127.3, 127.5, 127.7, 131.9, 138.1, 138.4, 141.1, 149.6, 150.5, 169.5, 173.7.

IR (film): ν = 3479 (w), 3365 (br w), 3057 (w), 2933 (w), 2835 (w), 1738 (s), 1676 (s), 1588 (m), 1511 (m), 1454 (m), 1243 (m), 1140 (m), 1021 (m), 743 (m) cm^{-1}.

HRMS (EI): calcd for $C_{37}H_{33}N_3O_7$ 631.2319; found 631.2314.

3b, $[\alpha]^{20}_D$ –4 (*c* = 0.1, MeOH).

^{1}H NMR (500 MHz, acetone-d_6): δ = 1.79 (d, *J* = 6.5 Hz, 3H), 2.24 (m, 4H), 3.50 (dd, *J* = 7.4, 14.1 Hz, 1H), 4.01 (s, 3H), 5.28 (s, 1H), 5.43 (q, *J* = 6.5 Hz, 1H), 7.15 (dd, *J* = 4.9, 7.4 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.44–7.50 (comp m, 2H), 7.63 (s, 1H), 7.79 (d, *J* = 8.3 Hz, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 8.19 (d, *J* = 7.7 Hz, 1H), 9.41 (d, *J* = 7.9 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 21.3, 23.4, 43.4, 53.2, 53.3, 53.4, 86.2, 86.5, 100.3, 109.2, 116.0, 117.6, 121.4, 122.7, 124.4, 125.3, 125.5, 125.7, 126.4, 127.4, 127.5, 130.1, 138.4, 139.7, 141.4, 171.6, 174.0.

IR (film): ν = 3407 (br w), 3053 (w), 2950 (w), 1733 (m), 1679 (s), 1450 (s), 1392 (s), 1308 (m), 1257 (m), 1199 (m), 1140 (m), 1083 (m), 748 (s) cm^{-1}.

HRMS (EI): calcd for $C_{28}H_{23}N_3O_5$ 481.1638; found 481.1637.

Acknowledgement

We are pleased to acknowledge the support of this investigation by the NIH (Grant 1RO1GM54131) and ACS (Grant DHP-82223). Bristol-Myers Squibb, Eli Lilly, Glaxo-Wellcome, Yamanouchi and Zeneca provided additional support through their Junior Faculty Awards programs. J.L.W is a fellow of the Alfred P. Sloan Foundation.

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Article Identifier:
1437-210X,E;1999,0,SI,1529,1533,ftx,en;C01799SS.pdf