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The Discovery of Small Molecule Carbamates as Potent Dual $\alpha_4\beta_1/\alpha_4\beta_7$ Integrin Antagonists

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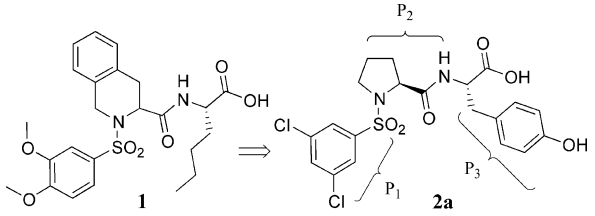
Abstract—The $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins are implicated in several inflammatory disease states. Systematic SAR studies of an $\alpha_4\beta_1$-specific arylsulfonyl-Pro-Tyr lead led to the identification of a new $\alpha_4\beta_7$ binding site, best captured by *O*-carbamates of Tyr for this structural class. Several compounds showed a 200- to 400-fold improvement in $\alpha_4\beta_7$ binding affinity while maintaining sub-nanomolar $\alpha_4\beta_1$ activity, for example **2l**, VCAM-Ig $\alpha_4\beta_1$ $IC_{50} = 0.13$ nM, VCAM-Ig $\alpha_4\beta_7$ $IC_{50} = 1.92$ nM.

Introduction

The integrins $\alpha_4\beta_1$ (very late antigen-4; VLA-4) and $\alpha_4\beta_7$ are heterodimeric cell surface glycoprotein transmembrane receptors that are divalent cation-dependent.[1] $\alpha_4\beta_1$ is expressed on all leukocytes except platelets and is a key mediator in cell–cell and cell–matrix interactions. As such, it mediates cell adhesion to vascular cell adhesion molecule-1 (VCAM-1), which is expressed on activated endothelial cells at sites of inflammation, and CS-1, an alternatively spliced form of fibronectin (Fn).[2] $\alpha_4\beta_7$ is expressed on leukocytes and is central to lymphocyte homing in the gastrointestinal tract via its interaction with the mucosal addressing cell adhesion molecule-1 (MAdCAM-1) expressed on endothelial cells of gut-associated mucosal tissues. $\alpha_4\beta_7$ also binds to VCAM-1 and Fn.[3]

Therapeutic efficacy in several animal models of inflammation and autoimmune diseases such as asthma, multiple sclerosis, and inflammatory bowel disease via the inhibition of the interaction between $\alpha_4\beta_1$ and/or $\alpha_4\beta_7$ and their ligands has been demonstrated using both α_4-antibodies and peptidyl antagonists.[4] Therefore, there is a substantial interest in developing small-molecule therapeutics for these disorders.[5]

Screening of a combinatorial library identified a capped dipeptide lead **1** ($\alpha_4\beta_1$ $IC_{50} = 58$ nM, $\alpha_4\beta_7$ $IC_{50} = 8330$ nM).[6] Initial derivatization efforts led to **2a** ($\alpha_4\beta_1$ $IC_{50} = 0.34$ nM, $\alpha_4\beta_7$ $IC_{50} = 820$ nM), a much more potent $\alpha_4\beta_1$ antagonist which also showed a somewhat increased activity versus $\alpha_4\beta_7$.[7,8] The three major binding elements, as determined by the SAR for this class of compounds, are designated P_1, an arylsulfonyl moiety, P_2, a prolyl residue, and P_3, a substituted phenylalanine, as shown in **2a**.[9a] We were intrigued by the possibility of locating additional binding sites to these receptors by elaboration of the tyrosine hydroxyl in **2a**. This investigation led to the identification of low nanomolar dual antagonists of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ which are reported herein.[9b]



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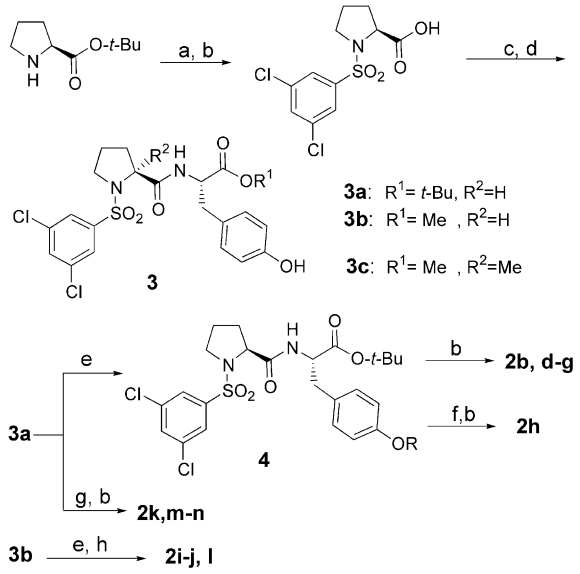
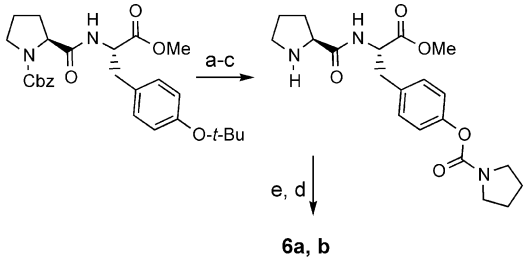
Chemistry

The first series of compounds studied are shown in Table 1. They were prepared from key intermediates **3a**,**b** (Scheme 1). Thus, sulfonylation of proline *t*-butyl ester followed by deprotection provided *N*-(3,5-dichlorobenzenesulfonyl)-proline.[10] This was coupled with the *t*-butyl ester or the methyl ester of tyrosine *t*-butyl ether. Liberation of the phenol via trifluoroacetic acid provided compounds **3a**,**b**.[11] Alkylation of **3a** with a variety of halides followed by removal of the *t*-butyl ester provided **2b**,**d**–**g**. For **2h**, tetrazole formation from **4**, where $R = CH_2CN$, preceded ester hydrolysis. Compounds **2i**–**j** and **l** were prepared from **3b** in an analogous manner. The esters **2k**,**m**–**n** were obtained by coupling of **3a** with the appropriate acids followed by ester hydrolysis.



Scheme 1. (a) $(3,5\text{-}Cl_2)C_6H_3SO_2Cl$, $NEt(iPr)_2$, DMAP, DCM, 94%; (b) 1/1 TFA/DCM, rt, 40–98%; (c) H-Tyr(*t*-Bu)-*O*(t-Bu or Me)·HCl, EDC, HOBt, NMM, DCM, rt, 80–85%; (d) excess 1/1 TFA/DCM, 0 °C, 2 h, 56–70%; (e) K_2CO_3, RX, DMF, 40 °C, 85–95%; (f) Me_3SnN_3, toluene, reflux, 20%; (g) EDC, DMAP, RCO_2H, DCM, rt, 90–97%; (h) 0.2 N NaOH/EtOH, rt, 40–70%.

Compounds **5a**–**i** (Table 2) were prepared via intermediate **3c** (obtained from α-methyl proline *t*-butyl ester instead of proline *t*-butyl ester in Scheme 1) or its (3-hydroxy)-Phe analogue, obtained by using (3-*t*-butoxy)-Phe methyl ester in the amide bond formation reaction



Scheme 2. (a) 1/1 TFA/DCM, rt, 70%; (b) K_2CO_3, $ClC(O)N(CH_2)_4$, DMF, 40 °C, 90%; (c) H_2, 10% Pd/C, MeOH, rt, quant; (d) 0.2 N NaOH/EtOH, rt 70–90%; (e) RSO_2Cl, $NEt(iPr)_2$, DMAP, DCM, 80–90%.

in Scheme 1 rather than (*O*-*t*-butyl)-tyrosine methyl ester. Subsequently, the target compounds were obtained using reaction sequences analogous to those shown for the preparation of **2i**–**j**,**l**.

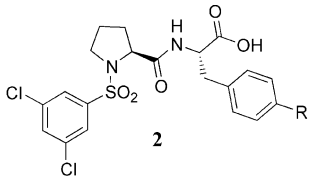
A slightly altered order of steps was used to prepare **6a**,**b** (Scheme 2). Here, the fully elaborated Pro-Tyr moiety (P_2–P_3) was assembled before modifications were made to obtain the desired P_1 unit.

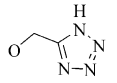
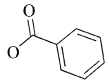
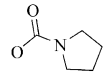
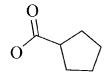
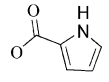
Biological Evaluations

The $\alpha_4\beta_1$ integrin binding affinity of the compounds reported was assessed by measuring the reduction in binding of ^{125}I-VCAM-Ig to a suspension of Jurkat cells (a human T Cell line $\alpha_4^+\beta_1^+\beta_7^-$) in the presence of the test compound, as previously described.[7] The assays are run in the presence of 1 mM of $MnCl_2$ and all compounds were tested at least in duplicate.

The $\alpha_4\beta_7$ integrin binding affinity of these compounds was determined in duplicate by a radioligand binding assay using ^{125}I-VCAM-Ig and a suspension of RPMI-8866 cells (a human B cell line $\alpha_4^+\beta_1^-\beta_7^+$) in the presence of 1 mM of $MnCl_2$, as described previously.[7]

Table 1. In vitro binding potencies of (*N*-arylsulfonyl)-prolyltyrosyl derivatives **2** versus ^{125}I-VCAM-Ig



Compd	R	$\alpha_4\beta_1$ IC_{50} (nM)	$\alpha_4\beta_7$ IC_{50} (nM)
2a	OH	0.34	820
2b	OCH_3	0.37	362
2c	O-*t*-(C_4H_9)	0.21	116
2d	$O(CH_2)_3CH_3$	1.11	299
2e	$OCH_2C_6H_5$	1.94	849
2f	$O(CH_2)_2OCH_3$	0.28	62.5
2g	OCH_2CN	0.26	246
2h		0.21	166
2i	OCH_2COCH_3	0.18	51.4
2j	OCH_2CO_2-*t*-(C_4H_9)	0.13	211
2k		0.45	43.2
2l		0.13	1.92
2m		0.22	58.6
2n		0.26	13

Results and Discussion

To begin, a series of simple alkyl ethers of the parent compound **2a** was evaluated (Table 1, **2a**–**e**).[12] The data suggest that these compounds generally bind well to $\alpha_4\beta_1$, although as the length and lipophilicity of the ether increased, the binding potency decreased (**2d**–**e** vs **2b**–**c**). The binding to the $\alpha_4\beta_7$ integrin was enhanced by steric bulk proximal to the phenolic oxygen (**2a** vs **2b**–**c**), although, in general, they remained modest.

Data from **2d** and **2f** show that isosteric replacement of an oxygen for a carbon atom provided a ~4-fold increase in potency towards both integrins, suggesting that an appropriately placed heteroatom may bring about substantial improvements to both the $\alpha_4\beta_1$ and $\alpha_4\beta_7$ binding. Placing this heteroatom in a more directional manner (**2g**) or the addition of multiple heteroatoms (**2h**) had little effect in the $\alpha_4\beta_1$ activity, but decreased the $\alpha_4\beta_7$ binding. Data from an alkoxy ketone and an alkoxy ester (**2i**–**j**) suggest that the requirements for good $\alpha_4\beta_7$ binding in this vicinity are much more limiting than those for the $\alpha_4\beta_1$ site and binding to the $\alpha_4\beta_7$ site appear to be sensitive to the spatial positioning of the hetero atom(s) incorporated.

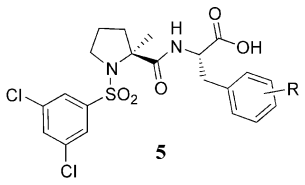
The effect of binding affinity induced by heteroatoms closer to the phenolic oxygen was studied. Data from **2k** and **2e** suggest that the presence of the carbonyl oxygen in the ester **2k** not only increases the $\alpha_4\beta_1$ binding by 4-fold but more importantly, it produces a 20-fold increase in the $\alpha_4\beta_7$ binding. The carbamate **2l** was prepared to study the effect of heteroatoms in 1,3-relationships with respect to the phenolic oxygen. This resulted in subnanomolar potency for $\alpha_4\beta_1$ and a 20- to 30-fold increase in potency for $\alpha_4\beta_7$ relative to the esters **2k** and **2m**. The data from **2k**,**m**–**n** versus **2l** show that the nitrogen of the carbamate plays a pivotal role in enhancing the $\alpha_4\beta_7$ activity by conferring a 20- to 30-fold increase in binding affinity. It also contributes a ~2-fold increase to the $\alpha_4\beta_1$ activity. Moving it to a neighboring position in the ring as in **2n** decreased the $\alpha_4\beta_7$ activity. Thus, the addition of appropriately arrayed oxygen and nitrogen proximal to the phenolic oxygen of **2a**, as in a carbamate (**2l**), can bring about a >400-fold increase in $\alpha_4\beta_7$ binding affinity and a 2-fold increase in $\alpha_4\beta_1$ activity.

The effect of α-alkylation of the prolyl moiety was examined (Table 2). Data from two pairs of compounds, **2c**/**5a**, and **2l**/**5b** show that the addition of the α-methyl group had little effect on the $\alpha_4\beta_1$ binding while the $\alpha_4\beta_7$ binding decreased by 3- to 6-fold. The scope and limitation of *N*-substituents on the carbamate are demonstrated by analogues **5b**–**f**. Data from the acyclic carbamates (**5c**–**e**) suggest that the $\alpha_4\beta_7$ binding is quite sensitive to steric congestion around the carbamate nitrogen while the $\alpha_4\beta_1$ binding is much less affected.

Several compounds with substituted (3-hydroxy)-Phe's at P_3 were prepared in an attempt to probe if either the $\alpha_4\beta_1$ or the $\alpha_4\beta_7$ potency-enhancing binding sites could be accessed from the 3-position (**5g**–**i**). The data from these carbamates show that for both integrins, particularly $\alpha_4\beta_7$, the more favorable receptor–ligand interaction projects from the phenolic position of Tyr rather than from the aromatic 3-position of Phe. Although in the case of **5h**, extending the length of the 3-substituent provided significant improvement in $\alpha_4\beta_1$ binding, suggesting that the longer chain was able to access partially the $\alpha_4\beta_1$ binding site.

A limited study was undertaken to probe the role of the *N*-arylsulfonyl substituent in $\alpha_4\beta_1$ and $\alpha_4\beta_7$ binding. As shown in Table 3, removal of the two chlorines in **2l** made

Table 2. In vitro binding potencies of (*N*-arylsulfonyl)-prolyltyrosine derivatives **5** versus ^{125}I-VCAM-Ig



5

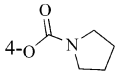
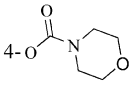
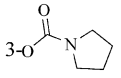
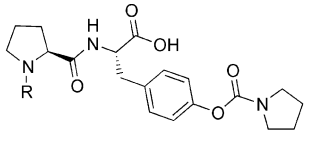
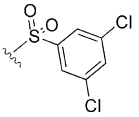
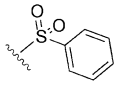
Compd	R	$\alpha_4\beta_1$ IC_{50} (nM)	$\alpha_4\beta_7$ IC_{50} (nM)
5a	4-*O*-*t*-(C_4H_9)	0.27	647
5b		0.17	5.26
5c	4-OC(O)N(CH_2CH_3)$_2$	0.19	29.8
5d	4-OC(O)N(*i*-Pr)$_2$	0.22	62.4
5e	4-OC(O)N(CH_3)(C_6H_5)	0.25	12.3
5f		0.13	3.51
5g	3-*O*-*t*-(C_4H_9)	1.79	Inactive @ 100 mM
5h	3-OCH_2CO_2-*t*-(C_4H_9)	0.41	Inactive @ 100 nM
5i		3.65	Inactive @ 100 nM

Table 3. In vitro binding potencies of prolyltyrosyl carbamates **6** versus ^{125}I-VCAM-Ig



6

Compd	R	$\alpha_4\beta_1$ IC_{50} (nM)	$\alpha_4\beta_7$ IC_{50} (nM)
21		0.13	1.92
6a		0.14	2.83
6b	SO_2CH_3	0.5	4.4

little difference in binding towards either integrin (**6a** vs **2l**). Replacing the aryl moiety by a methyl group produced a compound with a more balanced profile, mostly at the expense of increased $\alpha_4\beta_1$ binding (**6b** vs **6a**).

Thus, systematic SAR studies of the binding of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins to (*N*-arylsulfonyl)-prolyl-(*O*-substituted)-tyrosines revealed that an *O*-tyrosylcarbamate could confer a 300- to 400-fold increase to the $\alpha_4\beta_7$ binding affinity over that obtained from the parent tyrosyl derivative, yielding low nanomolar $\alpha_4\beta_7$ antagonists **2l**, **5f**, and **6a**. In this study, this $\alpha_4\beta_7$ binding site is reached most effectively by a carbamate moiety derived from the phenolic hydroxyl of P3-Tyr. This binding site is averse to steric congestion of the carbamate substituents. The $\alpha_4\beta_1$ integrin also accommodates this carbamate but the receptor–ligand interaction here is much less stringent in its spatial requirement of and tolerance for the positioning of various heteroatoms, enabling several different structural motifs to achieve a modest gain in potency.

The pharmacokinetic (PK) profile of several of the foregoing compounds were evaluated in rats (Table 4). Generally, these compounds exhibited moderate to high plasma clearance, low plasma half-life and poor oral absorption. The metabolic potential of the acidic moiety and the amide bond could both contribute to this type of PK profile, in addition to possible metabolic lability associated with carbamates of phenols.[13]

Table 4. Pharmacokinetic parameters[a] of selected compounds

Compd	Cl_p[b] (mL/kg/min)	$t_{1/2}$[c] (h)	F (%)
5b	27	ND[d]	6
5e	38	0.2	11
5f	50	ND	3
6a	176	0.2	ND

[a]Sprague–Dawley rats.
[b]Dose: 1 mg/kg iv; 2 mg/kg po (cassette dosing).
[c]$t_{1/2}$ = plasma half-life$_{(0-8h)}$.
[d]ND, not determined.

This investigation resulted in the discovery of highly potent small molecule dual $\alpha_4\beta_1/\alpha_4\beta_7$ integrin antagonists. Indeed, the dual antagonists identified from this study, for example, **6a** [$\alpha_4\beta_1$ (VCAM-Ig) $IC_{50} = 0.14$ nM, and $\alpha_4\beta_7$ (VCAM-Ig) $IC_{50} = 2.83$ nM] have been instrumental as tools for understanding the biochemistry of these integrins and their role in inflammatory processes.

Acknowledgement

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References and Notes

1. (a) Butcher, E. C. *Cell* **1991**, *67*, 1033. (b) Springer, T. A. *Cell* **1994**, *76*, 301. (c) Cox, D.; Aoki, T.; Seki, J.; Motoyama, Y.; Yoshida, K. *Med. Res. Rev.* **1994**, *14*, 195.
2. (a) Bevilacqua, M. P. *Annu. Rev. Immunol.* **1993**, *11*, 767. (b) Postigo, A. A.; Teixido, J.; Sanchez-Madrid, F. *Res. Immunol.* **1993**, *144*, 723.
3. (a) Berlin, C.; Berg, E. L.; Briskin, M. J.; Andrew, D. P.; Kilshaw, P. J.; Holzmann, I. L.; Weissman, A.; Hamann, A.; Butcher, E. C. *Cell* **1993**, *74*, 185. (b) Briskin, J. J.; McEvoy, L. M.; Butcher, E. C. *Nature* **1993**, *363*, 461. (c) Hamann, A.; Andrew, D. P.; Jablonski-Westrich, D.; Holzmann, B.; Butcher, E. C. *J. Immunol.* **1994**, *152*, 3282. (d) Bargatze, R. F.; Jutila, M. A.; Butcher, E. C. *Immunity* **1995**, *3*, 99.
4. (a) Lobb, R. R.; Hemler, M. E. *J. Clin. Invest.* **1994**, *94*, 1722. (b) Lin, K.-C.; Castro, A. C. *Curr. Opin. Chem. Bio.* **1998**, *2*, 453. (c) Molossi, S.; Elices, M.; Arrhenius, T.; Diaz, R.; Coulber, C.; Rabinovitch, M. *J. Clin. Invest.* **1995**, *95*, 2601. (d) Foster, C. A. *J. Allergy. Clin. Immunol.* **1996**, *98*, S270. (e) Elices, M. *Curr. Opin. Anti-Inflam. Immunomod. Invest. Drugs* **2000**, *2*, 228.
5. (a) Lobb, R. R.; Adams, S. P. *Exp. Opin. Invest. Drugs* **1999**, *8*, 935. (b) Adams, S. P.; Lobb, R. R. *Annu. Rep. Med. Chem.* **1999**, *34*, 179. (c) Engleman, V. W.; Kellogg, M. S.; Rogers, T. E. *Annu. Rep. Med. Chem.* **1996**, *31*, 191. (d) Shroff, H. N.; Schwender, C. F.; Dottavio, D.; Yang, L.-L.; Briskin, M. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2495. (e) Harriman, G. C. B.; Schwender, C. F.; Gallant, D.; Cochran, N. A.; Briskin, M. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1497.
6. Durette, P. L.; Hagmann, W. K.; MacCoss, M.; Mills, S. G.; Mumford, R. A.; Van Riper, G. M.; Schmidt, J. A.; Kevin, N. J. WO98/53814 A1, 1998; *Chem. Abstr.* **1999**, *130*, 52724m.
7. (a) Durette, P. L.; Hagmann, W. K.; MacCoss, M.; Mills, S. G.; Mumford, R. A. WO98/53818 A1, 1998; *Chem. Abstr.* **1999**, *130*, 33016r. (b) Durette, P. L.; Hagmann, W. K.; MacCoss, M.; Mills, S. G.; Mumford, R. A. WO98/53817 A1, 1998; *Chem. Abstr.* **1999**, *130*, 52736s.
8. (a) Several structurally similar compounds were described recently: Dappen, M. S.; Dressen, D. B.; Grant, F. S.; Konradi, A. W.; Pleiss, M. A.; Semko, C. M.; Thorsett, E. D.; Freedman, S. B.; Holsztynska, E. J.; Quinn, K. P.; Ashwell, S.; Banker, A. L.; Baudy, R. B.; Bicksler, J. J.; Giverson, J.; Leeson, P. D.; Lombardo, L. J.; Sarantakis, D. *Abstract of Papers, Part X*, 220th National Meeting of the American Chemical Society, Washington, DC, Aug. 20–24, 2000; American Chemical Society: Washington, DC, 2000; MEDI #135. (b) Thorsett, E. D.; Dappen, M. S.; Dressen, D. B.; Ellingboe, J. W.; Grant, F. S.; Jacobson, M.; Kincaid, S. L.; Konradi, A. W.; Kreft, A.; Lombardo, L. J.; Mann, W.; Nunn, D.; Pleiss, M. A.; Sabalski, J. E.; Sacchi, K.; Sarantakis, D.; Semko, C. M. *Abstract of Papers, Part X*, 220th National Meeting of the American Chemical Society, Washington, DC, Aug. 20–24, 2000; American Chemical Society: Washington, DC, 2000; MEDI #134.
9. (a) Hagmann, W. K.; Durette, P. L.; Lanza, T. J.; Kevin, N. J.; De Laszlo, S. E.; Kopka, I. E.; Young, D. N.; Magriotis, P. A.; Li, B.; Lin, L. S.; Yang, G. X.-Q.; Kamenecka, T.; Chang, L. L.; Wilson, J.; MacCoss, M.; Mills, S. G.; Van Riper, G.; McCauley, E. D.; Egger, L. A.; Kidambi, U.; Lyons, K. A.; Vincent, S. H.; Stearns, R. A.; Colleti, A. E.; Teffera, Y.; Tong, X.; Wang, J.; Zheng, S.; Owens, K. A.; Levorse, D. A.; Kim, P.; Schmidt, J. A.; Mumford, R. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2709. (b) For a study on substituted Phe's and related compounds at P3, see: Kopka, I. E.; Young, D.; Lin, L.; Mumford, R. A.; MacCoss, M.; Mills, S. G.; Van Riper, G.; McCauley, E.; Egger, L.; Kidambi, U.; Schmidt, J. A.; Lyons, K.; Stearns, R.; Vincent, S.; Colletti, A.; Wang, Z.; Tong, S.; Wang, J.; Zheng, S.; Owens, K.; Levorse, D.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* In press.
10. This and all other compounds prepared were characterized by mass spectrum (FAB or LC/MS), and 300, 400, or 500 MHz ^{1}H NMR.

11. A similar method has been described: Gibson, F. S.; Bergmeier, S. C.; Rapoport, H. *J. Org. Chem.* **1994**, *59*, 3216.
12. (a) *N*-Benzylpyroglutamyl-L-phenylalanines have been evaluated as VLA-4 receptor antagonists: Tilley, J. W.; Kaplan, G.; Rowan, K.; Schwinge, V.; Wolitzky, B. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1. (b) Chen, L.; Tilley, J. W.; Guthrie, R. W.; Mennona, F.; Huang, T.-N.; Kaplan, G.; Trilles, R.; Miklowski, D.; Huby, N.; Schwinge, V.; Wolitzky, B.; Rowan, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 729, and references cited therein.
13. (a) Hansen, K. T.; Faarup, P.; Bundgaard, H. J. *Pharm. Sci.* **1991**, *80*, 793. (b) Hansen, J.; Mørk, N.; Bundgaard, H. *Int. J. Pharm.* **1992**, *81*, 253.