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Tetrahedron Letters 44 (2003) 1571–1574

TETRAHEDRON LETTERS

Design and synthesis of conformationally constrained, extended and reverse turn pseudopeptides as Grb2-SH2 domain antagonists

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Received 5 December 2002; revised 20 December 2002; accepted 30 December 2002

Abstract—A series of conformationally constrained and flexible pseudopeptide derivatives of the tripeptide pYVN were prepared as potential antagonists of interactions of phosphotyrosine peptides with the Grb2-SH2 domain. The conformationally constrained compounds contained *trans*- and *cis*-cyclopropanes as replacements to enforce locally extended and reverse turn peptide conformations, respectively.

Small peptides bind to proteins in well-defined conformations, so there has been considerable interest in developing rigid replacements of peptide secondary structural elements for incorporation into non-peptidic analogues.[1] The rationale for introducing conformational constraints into flexible molecules owes its origin to the widely held belief that preorganizing such ligands into their biologically active conformations will provide analogues that have higher binding affinities as a consequence of a more favorable entropy of binding. Of course, there is the obvious caveat that such constraints must not introduce any bad contacts and that all the attractive interactions are maintained in the complex.[2]

We have explored the use of 1,2,3-trisubstituted cyclopropanes as rigid peptide replacements in designing novel enkephalin analogues as well as potent inhibitors of renin, HIV-1 protease, matrix metalloproteinases, and Ras farnesyltransferase.[3] The cyclopropane replacements in these compounds were designed to constrain the backbones and position the amino acid side chains in a predetermined orientations corresponding to the putative biologically active conformations of the related peptides. We recently conducted a detailed thermodynamic and structural study of the complexes of several Ac-pTyr-Glu-Glu-Ile derivatives bound to the Src-SH2 domain.[4] Somewhat surprisingly, we found that the $\Delta G_{binding}$ for flexible and constrained analogues of this tetrapeptide were approximately the same because the favorable entropic effect of restricting internal rotors was offset by unfavorable enthalpic effects that could not be attributed to specific ligand–protein interactions or lack thereof. Thus, introducing conformational constraints does not necessarily lead to higher affinity ligands, especially when entropy–enthalpy compensation intervenes.[5]

In order to explore the generality of entropy–enthalpy compensation in pseudopeptide–protein interactions, we were attracted to studying the binding of Ac-pTyr-Val-Asn-NH_2 (pYVN, **1**) and pseudopeptide derivatives thereof to the SH2 domain of the mammalian growth receptor bound protein 2 (Grb2). While the phosphotyrosine residue, pY, of pYVN binds to the Grb2-SH2 domain in an extended orientation, the ligand adopts an unusual type β-turn at the pY+1 position;[6] this structural feature has been imitated in the design of novel analogues.[7]

Preliminary modeling studies suggested that the cyclopropane rings in **2** and **3** should mimic the salient three-dimensional structural features of **1** when bound to the Grb2-SH2 domain. Compounds **4** and **5** would then serve as flexible controls of **2** and **3**, respectively. The cyclopropane in **2**, which enforces a local extended conformation on the peptide backbone of the pY residue, is derived by replacing the amide nitrogen atom of the native tripeptide **1** with a carbon atom and connecting this atom to C(β) of the tyrosine side chain

Keywords: peptide mimics; conformational constraints; cyclopropanes; SH2 domain.

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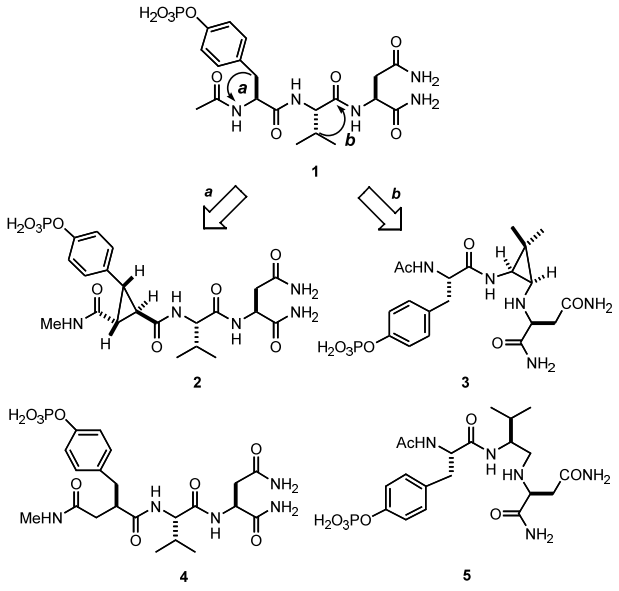
0040-4039/03/$ - see front matter
PII: S0040-4039(03)00013-3

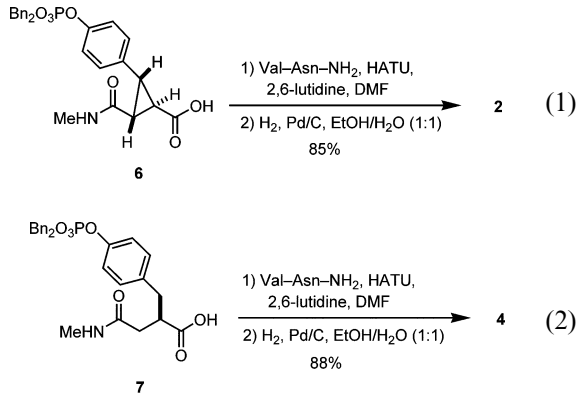
(Scheme 1, mode *a*). Examination of X-ray structures of complexes of the Grb2-SH2 domain and peptide-derived ligands reveals that the *N*-terminal amide of **1** is not likely involved in interactions with the SH2 domain.[6,7] Nevertheless, we wanted to preserve the *N*-terminal functional character of **1** in our ligand design, so a *N*-methyl carboxamide group was placed on the cyclopropane ring of **2**.

The cyclopropane in **3**, which is proposed to favor a reverse turn in the backbone at the pY+1 position, arises from replacing the carbonyl carbon atom on **1** with a sp^3-carbon and linking this atom to C(β) of the valine side chain (Scheme 1, mode *b*). Based upon previous work in our labs,[3g,i] we were cognizant of the potential liabilities of replacing an amide bond with an amino linkage when the amide group itself is in some way involved in binding. However, because neither the CO of Val nor the NH of Asn in **1** interact directly with the Grb-SH2 domain, it seemed reasonable to assess the impact of an aminoethyl substitution in this system. If a basic nitrogen atom were highly detrimental to binding, second generation ligands could be designed in which an ether function would link the Asn and Val residues. The geminal dimethyl groups on the cyclopropane ring of **3** would not occupy precisely the same positions as the methyl groups in **1**, but initial modeling suggested they should not incur unfavorable interactions with the SH2 domain. We now report the syntheses and preliminary biological evaluation of **2**–**5** as analogues of **1**.

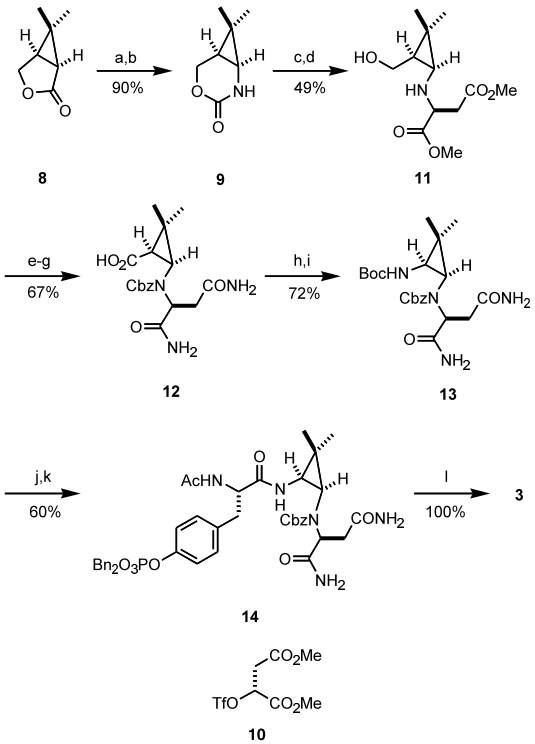
Compounds **2** and **4** were prepared as single isomers by coupling the known acids **6** and **7**[4,8] with the dipeptide H-Val-Asn-NH_2 in the presence of HATU, followed by global removal of the benzyl protecting groups by catalytic hydrogenolysis (Eqs. (1) and (2)).[9,10]



Scheme 1.



Synthesis of the constrained pseudopeptide **3** commenced with the hydrazinolysis of the known lactone **8**[11] followed by a Curtius reaction of the resultant hydrazide that led to the cyclic carbamate **9** (Scheme 2). Base-induced hydrolysis of the carbamate moiety in **9** furnished an amino alcohol that was *N*-alkylated with the triflate **10**, which was prepared in situ from (*R*)-dimethyl malate, to give **11** as the only isolated stereoisomer in 49% overall yield.[12] Reaction of **11** with NH_3 in MeOH in the presence of a catalytic amount of NaCN delivered a diamide that was transformed into **12** via *N*-protection and oxidation of the primary alcohol function. One-carbon degradation of the carboxyl group in **12** via a modified Curtius reaction led to the protected diaminocyclopropane **13**.[13] The *N*-Boc pro-

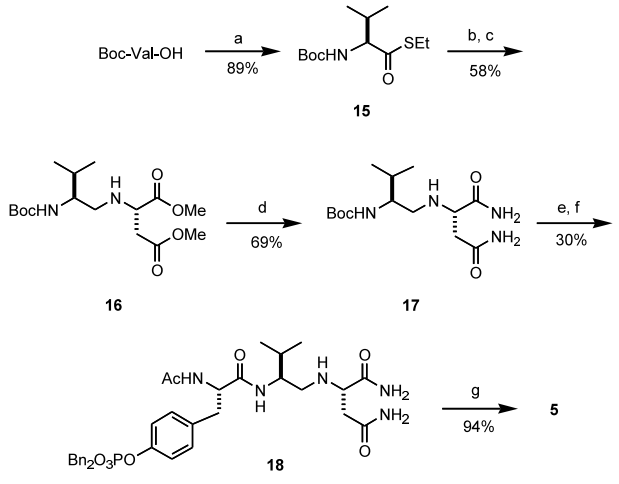


Scheme 2. *Reagents and conditions*: (a) $H_2NNH_2{\cdot}H_2O$, MeOH; (b) HONO, Et_2O/H_2O, 0°C, then toluene, 80°C; (c) $Ba(OH)_2$, aq. dioxane; (d) **10**, 2,6-lutidine, *i*-Pr_2NEt, CH_2Cl_2, 0°C to rt; (e) NH_3, NaCN (cat.), MeOH, 50°C; (f) Cbz-Cl, *i*-Pr_2NEt, THF, CH_3CN, Δ; (g) $RuCl_3$, $NaIO_4$, $NaHCO_3$, $CH_3CN/CCl_4/H_2O$; (h) EtOCOCl, Et_3N, 0°C, NaN_3; (i) *t*-BuOH, Δ; (j) CF_3CO_2H; (k) HATU, Ac-pY*-OH, 2,6-lutidine, DMF, −10°C to rt; (l) H_2, Pd/C, EtOH.

tecting group was removed using CF_3CO_2H, and the resultant unstable amine was immediately coupled with *N*-acetyl-L-Tyr(PO_3Bn_2)-OH (Ac-pY*-OH)[14] using HATU to give the protected tripeptide **14** as a single isomer in 60% overall yield. Global deprotection by hydrogenolysis gave **3** in virtually quantitative yield.[15]

The synthesis of the flexible analogue **5** began with converting Boc-valine into the thioester **15** with DCC and EtSH in 89% yield (Scheme 3). The thioester moiety of **15** was reduced using the Fukuyama protocol[16] to give an aldehyde that was then coupled with L-aspartic acid dimethyl ester hydrochloride by reductive amination using $NaBH(OAc)_3$ to produce **16** as a single diastereomer.[17] The amine **16** thus obtained was isolated as a single diastereomer in 58% yield over the two steps. The ester groups in **16** were transformed into their respective amides using NH_3 in the presence of NaCN as before to provide **17**. Acid-catalyzed removal of the *N*-Boc protecting group and coupling the resultant amine with Ac-pY*-OH in the presence of HATU afforded the protected tripeptide **18** as a single isomer in 30% overall yield. Complete removal of the benzyl protecting groups by catalytic hydrogenolysis in the presence of HCl then delivered **5**.[18]

We have determined the binding constants, K_a (M^{-1}) of **1**–**5** with the Grb2-SH2 domain in a series of preliminary studies as follows: **1**, $4.9(\pm0.4)\times10^5$; **2**, $5.2(\pm0.7)\times10^5$; **3**, $<1\times10^3$; **4**, $2.9(\pm0.7)\times10^5$; and **5**, $2.0(\pm0.5)\times10^4$.[19] Thus, analogues of **1** with conformationally constrained and flexible replacements of a phosphotyrosine residue (e.g. **2** and **4**) exhibit similar binding affinities. Based upon these data, it seems likely that the orientations of the substituents on the cyclopropane ring in **2** mimics the bound conformation of the corresponding groups in **1** and **4**. On the other hand, **3** and **5**, which contain rigid and flexible replacements of the valine residue in **1**, have significantly lower binding affinities. Comparing the binding of **4** and **5** suggests that introducing an amino linkage between Asn and Val is somewhat detrimental. However, the nature of the substitution on cyclopropane ring in **3** also has a negative impact on binding, and further variations of the cyclopropane-derived replacement in analogues of **1** must be evaluated in order to ascertain whether a cyclopropane ring may stabilize a turned structure.



Scheme 3. *Reagents and conditions*: EtSH, DCC, DMAP, CH_2Cl_2, 30 min; (b) Et_3SiH, Pd/C (10%), CH_2Cl_2, 30 min; (c) $Na(OAc)_3BH$, L-aspartic acid dimethyl ester hydrochloride, DMF, 15 min; (d) NH_3, NaCN (cat.), MeOH, 50°C, 3 d; (e) CF_3CO_2H, 1.5 h; (f) HATU, Ac-pY*-OH, 2,6-lutidine, DMF, −10°C to rt, 12 h; (g) H_2, HCl, Pd/C, EtOH, 2 h.

A more detailed analysis of the energetic parameters ΔH and ΔS associated with the binding of these and other related ligands to the Grb2-SH2 domain and X-ray crystallographic studies of selected complexes are the subjects of ongoing studies. The results of these investigations will be reported in due course.

Acknowledgements

We thank the National Institutes of Health, The Robert A. Welch Foundation, the Texas Advanced Research Program, Pfizer, Inc. and Merck Research Laboratories for their generous support of our research. T.B.S. and A.R.W. are also grateful to Pfizer, Inc. for support by Summer Undergraduate Research Fellowships. We are also thankful to Dr. James P. Davidson for helpful discussions.

References

1. For reviews of constrained peptide mimics, see: (a) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244–1267; (b) Gante, J. *Angew. Chem., Int. Ed. Eng.* **1994**, *33*, 1699–1720; (c) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789–12854; (d) Goodman, M.; Zhang, J. *Chemtracts-Org. Chem.* **1997**, *10*, 629–645.
2. (a) DeGrado, W. F. *Adv. Protein Chem.* **1988**, *39*, 51–124; (b) Kahn, M. *Synlett* **1993**, 821–826; (c) Olson, G. L.; Bolin, D. R.; Bonner, M. P.; Bös, M.; Cook, C. M.; Fry, D. C.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusieck, V. K.; Sarabu, R.; Sepinwall, J.; Vincent, J. P.; Voss, M. E. *J. Med. Chem.* **1993**, *36*, 3039–3059; (d) Schneider, J. P.; Kelley, J. W. *Chem. Rev.* **1995**, *95*, 2169–2187.
3. (a) Martin, S. F.; Austin, R. E.; Oalmann, C. J.; Baker, W. R.; Condon, S. L.; DeLara, E.; Rosenberg, S. H.; Spina, K. P.; Stein, H. H.; Cohen, J.; Kleinert, H. D. *J. Med. Chem.* **1992**, *35*, 1710–1721; (b) Baker, W. R.; Jae, H.-S.; Martin, S. F.; Condon, S. L.; Stein, H. H.; Cohen, J.; Kleinert, H. D. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1405–1410; (c) Martin, S. F.; Oalmann, C. J.; Liras, S. *Tetrahedron* **1993**, *49*, 3521–3532; (d) Martin, S. F.; Dorsey, G. O.; Gane, T. H.; Hillier, M.; Kessler, H.; Baur, M.; Mathä, B.; Erickson, J. W.; Bhat, T. N.; Munshi, S.; Gulnik, S. V.; Topol, I. A. *J. Med. Chem.* **1998**, *41*, 1581–1597; (e) Hillier, M. C.; Martin, S. F. In *Methods in Molecular Medicine: Vol. 30, Peptidomimetics Protocols*; Kazmierski, W. M.; Walker, J. M., Eds.; Humana Press: Totowa, NJ, 1999; pp. 397–406; (f)

Dwyer, M. P.; Martin, S. F. In *Methods in Molecular Medicine: Vol. 30, Peptidomimetics Protocols*, Kazmierski, W. M.; Walker, J. M., Eds.; Humana Press: Totowa, NJ, 1999; pp. 407–416; (g) Martin, S. F.; Dwyer, M. P.; Hartmann, B.; Knight, K. S. *J. Org. Chem.* **2000**, *65*, 1305–1318; (h) Hillier, M. C.; Davidson, J. P.; Martin, S. F. *J. Org. Chem.* **2001**, *66*, 1657–1671; (i) Reichelt, A.; Gaul, C.; Frey, R. R.; Kennedy, A.; Martin, S. F. *J. Org. Chem.* **2002**, *67*, 4062–4075.

4. Davidson, J. P.; Lubman, O.; Rose, T.; Waksman, G.; Martin, S. F. *J. Am. Chem. Soc.* **2002**, *124*, 205–215.
5. (a) Sharp, K. *Protein Sci.* **2001**, *10*, 661–667; (b) Liu, L.; Guo, Q.-X. *Chem. Rev.* **2001**, *101*, 673–696; (c) Dunitz, J. D. *Chem. Biol.* **1995**, *2*, 709–712.
6. Rahuel, J.; Gay, B.; Erdmann, D.; Strauss, A.; Garcia, E.; Furet, P.; Caravatti, G.; Fretz, H.; Schoepfer, J.; Gruetter, M. G. *Nature Struct. Biol.* **1996**, *3*, 586–589.
7. (a) Ettmayer, P.; France, D.; Gounarides, J.; Jarosinski, M.; Martin, M.-S.; Rondeau, J.-M.; Sabio, M.; Topiol, S.; Weidmann, B.; Zurini, M.; Bair, K. W. *J. Med. Chem.* **1999**, *42*, 971–980; (b) Garcia-Echeverria, C.; Gay, B.; Rahuel, J.; Furet, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2915–2920; (c) Gao, Y.; Wei, C.-Q.; Burke, T. R., Jr. *Org. Lett.* **2001**, *3*, 1617–1620.
8. Davidson, J. P.; Martin, S. F. *Tetrahedron Lett.* **2000**, *41*, 9459–9464.
9. The structure assigned to each compound is in complete accord with its spectral (^{1}H and ^{13}C NMR, IR, mass) characteristics; molecular compositions of all new compounds were determined by high resolution mass measurements of purified materials. All yields are based on isolated, purified materials judged >95% pure by ^{1}H NMR spectroscopy.
10. ^{1}H NMR spectrum of **2** (500 MHz, D_2O): δ 7.27 (d, $J=8.7$ Hz, 2 H), 7.12 (d, $J=7.4$ Hz, 2 H), 4.73–4.70 (m, 1 H), 4.13 (d, $J=6.9$ Hz, 1 H), 2.96–2.94 (m, 1 H), 2.87–2.84 (m, 2 H), 2.76–2.74 (m, 1 H), 2.60 (s, 3 H), 2.54 (dd, $J=10.0$, 4.8 Hz, 1 H), 2.17–2.08 (m, 1 H), 0.99 (d, $J=8.1$ Hz, 3 H), 0.97 (d, $J=8.1$ Hz, 3 H). ^{1}H NMR spectrum of **4** (500 MHz, D_2O): δ 7.17–7.09 (comp m, 4 H), 4.57 (dd, $J=7.7$, 6.1 Hz, 1 H), 3.96 (d, $J=7.8$ Hz, 1 H), 3.13–3.08 (m, 1 H), 2.81–2.77 (comp m, 3 H), 2.68–2.65, (m, 3 H), 2.64 (s, 3 H), 2.53–2.41 (comp m, 2 H), 1.93 (app hep, $J=6.8$ Hz, 1 H), 0.85 (d, $J=6.8$ Hz, 6 H).
11. Doyle, M. P.; Austin, R. E.; Bailey, A. S.; Dwyer, M. P.; Dyatkin, A. B.; Kalinin, A. V.; Kwan, M. M. Y.; Liras, S.; Oalmann, C. J.; Pieters, R. J.; Protopopva, M. N.; Raab, C. E.; Roos, G. H. P.; Zhou, Q.-L.; Martin, S. F. *J. Am. Chem. Soc.* **1995**, *117*, 5763–5775.
12. For related *N*-alkylations, see: Chapman, K. T.; Kopka, I. E.; Durette, P. L.; Esser, C. K.; Lanza, T. J.; Izquierdo-Martin, M.; Niedzwiecki, L.; Chang, B.; Harrison, R. K.; Kuo, D. W.; Lin, T.-Y.; Stein, R. L.; Hagmann, W. K. *J. Med. Chem.* **1993**, *36*, 4293–4301. See also Refs. 3g,i.
13. Englert, H. C.; Gerlach, U.; Goegelein, H.; Hartung, J.; Heitsch, H.; Mania, D.; Scheidler, S. *J. Med. Chem.* **2001**, *44*, 1085–1098.
14. Llinas-Brunet, M.; Beaulieu, P. L.; Cameron, D. R.; Ferland, J.-M.; Gauthier, J.; Ghiro, E.; Gillard, J.; Gorys, V.; Poirier, M.; Rancourt, J.; Wernic, D.; Betageri, R.; Cardozo, M.; Jakes, S.; Lukas, S.; Patel, U.; Proudfoot, J.; Moss, N. *J. Med. Chem.* **1999**, *42*, 722–729.
15. ^{1}H NMR spectrum of **3** (500 MHz, D_2O): δ 7.22 (d, $J=8.5$ Hz, 2 H), 7.19 (d, $J=8.5$ Hz, 2 H), 4.52–4.49 (m, 1 H), 3.52–3.49 (m, 1 H), 3.14–2.89 (comp m, 2 H), 2.62 (dd, $J=15.0$, 5.7, 1 H), 2.53 (dd, $J=15.0$, 8.0 Hz, 1 H), 2.30 (d, $J=6.0$ Hz, 1 H), 2.00 (d, $J=6.0$ Hz, 1 H), 1.95 (s, 3 H), 1.03 (s, 3 H), 0.93 (s, 3 H).
16. (a) Fukuyama, T.; Lin, S.; Li, L. *J. Am. Chem. Soc.* **1990**, *112*, 7050–7051; (b) Han, Y.; Chorev, M. *J. Org. Chem.* **1999**, *64*, 1972–1978.
17. Based upon ^{1}H NMR evidence, no epimerization occurred at the carbon atom bearing the isopropyl group during the conversion of **15** into **16**. The reaction sequence was performed with racemic **15** to establish that the two epimers could be distinguished by ^{1}H NMR.
18. ^{1}H NMR spectrum of **5** (500 MHz, D_2O): δ 7.27 (d, $J=8.2$ Hz, 2 H), 7.17 (d, $J=8.2$ Hz, 2 H), 4.57 (dd, $J=8.4$, 7.0 Hz, 1 H), 4.26 (dd, $J=7.7$, 4.9 Hz, 1 H), 3.98–3.94 (m, 1 H), 3.21 (dd, $J=12.9$, 4.3 Hz, 1 H), 3.13 (dd, $J=13.9$, 7.0 Hz, 1 H), 3.05–2.97 (m, 2 H), 2.95–2.87 (m, 2 H), 1.96 (s, 3 H), 1.90–1.84 (m, 1 H), 0.88 (dd, $J=16.9$, 6.8 Hz, 6 H).
19. Binding constants were determined using isothermal titration calorimetry following the procedure outlined in: McNemar, C.; Snow, M. E.; Windsor, W. T.; Prongay, A.; Mui, P.; Zhang, R.; Durkin, J.; Le, H. V.; Weber, P. C. *Biochemistry* **1997**, *36*, 10006–10014.