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Syntheses of Conformationally Constricted Molecules as Potential NAALADase/PSMA Inhibitors

Pingyu Ding, Marvin J. Miller,* Yi Chen, Paul Helquist, A. Jayne Oliver, and Olaf Wiest

Walther Cancer Research Center & Department of Chemistry & Biochemistry, University of Notre Dame, 251 Nieuwland Science Hall, Notre Dame, Indiana 46556

mmiller1@nd.edu

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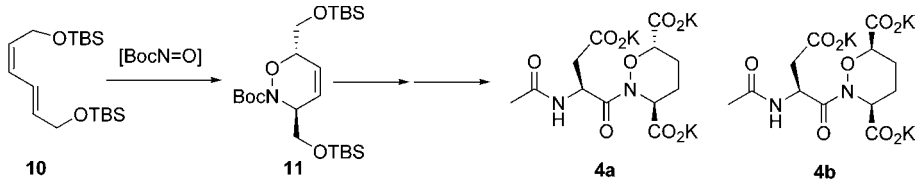
ABSTRACT



Two six-membered ring targeted analogues of PSMA inhibitors (4a and 4b) were designed on the basis of a computational analysis and synthesized. (*E*,*Z*)-Diene 10 was subjected to the nitroso Diels–Alder reaction to give the 1,4-trans six-membered ring adduct, 4a. The *cis* isomer 4b was derived from similar nitroso cycloaddition reactions with the correspnding (*E*,*E*)-diene and separately from cyclohexadiene. The IC_{50} values of 4a and 4b in a NAALADase assay were found to be 0.9 and 0.1 μM, respectively.

The development of new drugs for the treatment of cancer is increasingly based upon detailed molecular level understanding of the function of malignant cells. Breakthroughs in molecular and cell biology have led to the discovery of several differences between normal and abnormal cells. These differences provide a basis for designing new compounds that are able to differentiate normal cells from cancer cells and thus serve as a means to both diagnose and treat malignancies.

Prostate cancer affects approximately 180 000 men and kills 40 000 men in the United States each year, primarily due to the continuing growth of androgen-independent prostate cancer disseminated throughout the body. In most individuals, prostate cancer is proliferatively quiescent, with typically less than 5% of the cells proliferating per day. This renders most traditional chemotherapies ineffective since they typically affect proliferating cells. A promising target for diagnosis and treatment of prostate cancer is prostate specific membrane antigen (PSMA), which unlike the better known prostate-specific antigen (PSA) is a membrane-bound protein that is expressed exclusively by prostate tumor cells.[1] PSMA is a 110 KDa type II transmembrane protein. It is highly homologous to the neuropeptidase NAALADase (*N*-acetyl-α-linked acidic dipeptidase) that releases *N*-acetyl aspartate (**2**) and the neurotransmitter, glutamate (**3**), from both the neuronal peptide *N*-acetylaspartylglutamate (NAAG, **1**) and folate polyglutamate. Although the structure of PSMA has not yet been elucidated, a homology model of NAALADase has been described.[2]

The design, syntheses, and biological studies of inhibitors of NAALADase activity have led to compounds that show considerable therapeutic promise.[3] **VA-033** (IC_{50} = 12.5 nM; Figure 1) is a representative compound assayed by Tennis-

(1) (a) Carter, R. E.; Feldman, A. R.; Coyle, J. T. *Proc. Natl. Acad. Sci.* **1996**, *93*, 749. (b) Luthi-Carter, R.; Barczak, A. K.; Speno, H.; Coyle, J. T. *J. Pharm. Exp. Ther.* **1998**, *286*, 1020. (c) O'Keefe, D. S.; Su, S. L.; Bacich, D. J.; Horiguchi, Y.; Luo, Y.; Powell, C. T.; Zandvliet, D.; Ressell, P. J.; Molloy, P. L.; Nowak, N. J.; Shows, T. B.; Mullins, C.; Vonderhaar, R. A.; Fair, W. R.; Heston, W. D. W. *Biochim. Biophys. Acta* **1998**, *1443*, 113. (d) Pangalos, M. N.; Neffs, J. M.; Somers, M.; Verhasselt, P.; Bekkers, M.; van der Helm, L.; Fraiponts, E.; Ashton, D.; Gordon, R. D. *J. Biol. Chem.* **1999**, *274*, 8470. (e) Passani, L. A.; Vonsattel, J. P. J.; Carter, R. E.; Coyle, J. T. *Mol. Chem. Neuropath.* **1997**, *31*, 97. (f) Tiffany, C. W.; Lapidus, R. G.; Merion, A.; Calvin, D. C.; Slusher, B. S. *Prostate* **1999**, *39*, 28.

(2) Rong, S.-B.; Zhang, J.; Neale, J. H.; Wroblewski, J. T.; Wang, S.; Kozikowski, A. P. *J. Med. Chem.* **2002**, *45*, 4140.

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wood's group and shown to have high activity.[4] This compound serves as a potent inhibitor of the NAALADase activity associated with PSMA that is expressed on LNCap human prostate cancer cells and by tumor cells *in vivo*. A computational analysis of the complete dataset of Tenniswood et al. revealed a strong correlation between the number of thermally accessible conformations as determined by Monte Carlo simulations using the MMFF method and IC_{50} values in a NALAADase assay.[5] This was rationalized by a large entropic penalty for the binding of highly flexible compounds. Cyclic, conformationally restricted inhibitors such as **4** were therefore predicted to be more active than their acyclic analogues. It was further hypothesized that a low RMS for deviations in preferred conformations between a given compound and the highly active **VA-033** would indicate analogous binding interactions and a highly active compound. On the basis of conformational studies of **4**, it was thus predicted that the six-membered cyclic analogues with a *trans* configuration of the carboxylic acid groups (**4a**, $n = 2$) would be the most active. This compound provides a restricted structure with only 22 conformations within 3 kcal/mol of the global minimum. Both carboxylic acid groups required for binding within the specificity pocket of the active site of NAALADase are positioned in pseudoequatorial positions, and the compound has a small RMS deviation from that of the lowest energy conformation of **VA-033**. To provide experimental support for the calculationally predicted effect of conformation on biological activity, we hereby describe the design, syntheses, and studies of both the *trans* and *cis* six-membered ring compounds, **4a** and **4b** ($n = 2$).

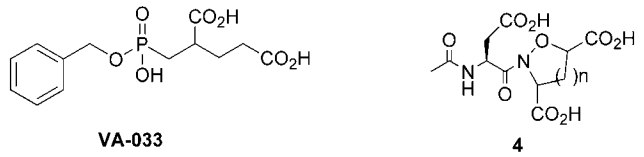
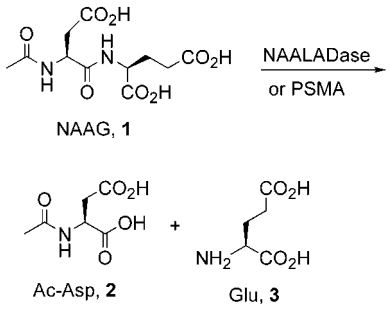


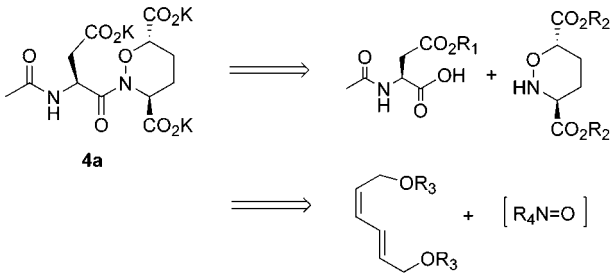
Figure 1. Known PSMA inhibitor and designed inhibitors.

For the synthesis of the *trans* product **4a** ($n = 2$), the key step was the construction of the *trans*-disubstituted six-membered ring nitroso Diels−Alder cycloadduct as shown in Scheme 2. We expected the low reactivity of the (*E*,*Z*)-diene to be compensated by the highly reactive dienophilic acyl nitroso species.

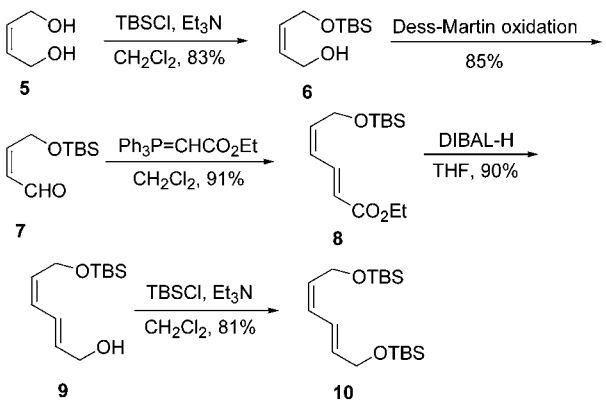
Scheme 1



Scheme 2. Retrosynthetic Analysis of Six-Membered *trans* Product **4a**



Scheme 3. Synthesis of (*E*,*Z*)-Diene **10**



As shown in Scheme 3, (*E*,*Z*)-diene **10** was prepared in five steps in 46% overall yield. Thus, commercially available *cis*-diol **5** was monoprotected as a TBS ether and then oxidized to the corresponding aldehyde with Dess−Martin reagent.[6] The resulting aldehyde was transformed into ester **8** via a Wittig reaction. Subsequent reduction and protection provided the desired (*E*,*Z*)-diene **10**, which turned out to be unstable; it was observed to slowly polymerize at room temperature. However, it could be stored at −20 °C for a few months without significant polymerization.

(*E*,*Z*)-Diene **10** reacted with an acylnitroso moiety, generated *in situ* from oxidation of *tert*-butyl *N*-hydroxycarbamate with sodium periodate, at 0 °C to provide a ca. 1:1 mixture of *cis* and *trans* adducts in less than 20% overall yield.[7] Using tetra-*n*-butylammonium periodate as the oxidizing reagent

(3) (a) Vitharana, D.; France, J. E.; Scarpetti, D.; Bonneville, G. W.; Majer, P.; Tsukamoto, T. *Tetrahedron: Asymmetry* **2002**, *12*, 1609. (b) Jackson, P. F.; Tays, K. L.; Maclin, K. M.; Ko, Y.-S.; Li, W.; Vitharana, D.; Tsukamoto, T.; Stoermer, D.; Lu, X.-C. M.; Wozniak, K.; Slusher, B. S. *J. Med. Chem.* **2001**, *44*, 4170. (c) Jackson, P. F.; Cole, D. C.; Slusher, B. S.; Stetz, S. L.; Ross, L. E.; Donzanti, B. A.; Trainor, D. A. *J. Med. Chem.* **1996**, *39*, 619. (d) Slusher, B. S.; Vornov, J. J.; Thomas, A. G.; Hurn, P. D.; Harukuni, I.; Bhardwaj, A.; Traystman, R. J.; Robinson, M. B.; Britton, P.; Lu, X. C. M.; Tortella, F. C.; Wozniak, K. M.; Yudkoff, M.; Potter, B. M.; Jackson, P. F. *Nat. Med.* **1999**, *5*, 1396.

(4) Tang, H.; Brown, M.; Ye, Y.; Huang, G.; Zhang, Y.; Wang, Y.; Zhai, H.; Chen, X.; Shen, T. Y.; Tenniswood, M. *Biochem. Biophys. Res. Commun.* **2003**, *307*, 8.

(5) (a) Oliver, A. J.; Wiest, O.; Helquist, P.; Miller, M. J.; Tenniswood, M. *Bioorg. Med. Chem.* **2003**, *11*, 4455. (b) Oliver, A. J.; Wiest, O.; Helquist, P.; Miller, M. J. Submitted for publication.

in CH_2Cl_2 did not improve the yield. However, when the initial reaction temperature was decreased to −10 °C, the overall yield improved to 50%. It was interesting to observe that all of the (*E*,*Z*)-diene **10** had been consumed during the reaction. Only (*E*,*E*)-diene **13** was isolated with these two adducts. The two Diels−Alder adducts could be carefully separated by column chromatography. However, it was difficult to differentiate the *cis* and *trans* adducts only by comparison of the coupling constants in the NMR spectra. For the purpose of structure assignment, we synthesized the *cis* adduct **12** by using the known (*E*,*E*)-diene **13** as starting material.[8] Using sodium periodate as the oxidizing agent in methanol−water only provided traces of product with most of the diene being recovered due to the poor solubility of (*E*,*E*)-diene **13** in methanol−water. However, employing tetra-*n*-butylammonium periodate as the oxidizing agent in CH_2Cl_2 led to the formation of adduct **12** in 68% yield with recovered starting material. The structure of **12** was confirmed by comparison of the NMR spectra of the products from the two cycloaddition reactions. It became apparent that the unstable (*E*,*Z*)-diene **10** was transformed into the more stable (*E*,*E*)-diene **13** under the above reaction conditions. The mechanistic details for the isomerization still need to be explored in further studies.

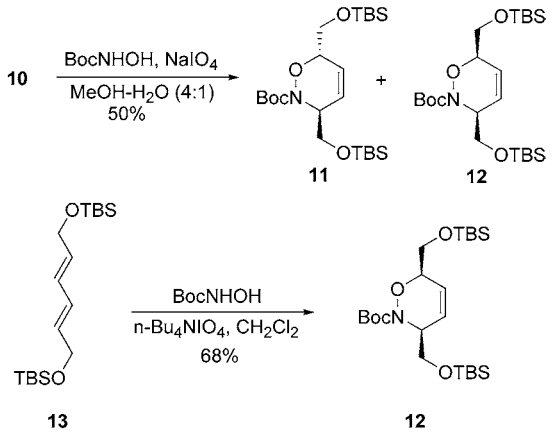
The TBS protecting groups were removed by TBAF in THF to provide olefin diol **14**, which was subjected to hydrogenation to reduce the double bond. Pd−C was first employed as the catalyst, which we found to be problematic due to a lack of reproducibility. We then turned to the use of $ClRu(PPh_3)_3$ as a catalyst,[9] and obtained more reproducible results. However, the catalyst could not be easily separated by column chromatography from the product. Thus, the crude material was used directly for the next step without purification.

Early efforts to oxidize diol **14** to the dialdehyde using Dess−Martin, PCC, and Swern oxidation conditions failed. We then attempted to oxidize the diol to the corresponding diacid through a one-step approach using $RuCl_3$−$NaIO_4$ as the oxidizing agent.[10] The reaction proceeded well, and the diacid was readily obtained. This method provided a one-step approach to the diacid from the corresponding diol. Due to difficulty of purification of the resulting diacid, the crude diacid was treated with *N*,*N*′-dicyclohexyl-*O*-benzylisourea in toluene at 100 °C for 2 h to provide dibenzyl ester **15** in 46% overall yield over three steps.[11]

Diester **15** was subsequently subjected to Boc deprotection by treatment with TFA in CH_2Cl_2 at room temperature to give the corresponding free amine **16**. A variety of coupling methods (HOAt/EDC, HOBt/EDC, and DCC/DMAP) were unsuccessful in an effort to synthesize **17** from **16** and *N*-acetyl-*β*-*tert*-butyl-L-aspartic acid. However, the use of isobutyl chloroformate and *N*-methylmorpholine as coupling reagents afforded **17** smoothly as an inseparable mixture of diastereomers.[12] Removal of the *tert*-butyl group by treatment with TFA, followed by hydrogenolytic cleavage of the benzyl protecting groups and ion exchange chromatography (Dowex, K^+), gave the *trans*-potassium salt **4a** as a diastereomeric mixture in 75% yield.[13]

The unambiguous synthesis of the *cis* compound **4b** was completed as shown in Scheme 6. Diels−Alder adduct **18** was treated with TFA to provide free amine **19**,[14] which was coupled with suitably protected aspartic acid using EDC and HOAt as coupling reagents in CH_3CN to afford **20** in 75% yield.[15] Then, the Boc group was removed, and the resulting free amine was treated with acetic anhydride in the presence of pyridine in CH_2Cl_2 to give **21** in excellent yield. Oxidative cleavage of the double bond of **21** with $KMnO_4$ in the

Scheme 4. Acylnitroso Diels−Alder Reaction and Structural Assignment of Products



(6) (a) Kruger, A. W.; Meyers, A. I. *Tetrahedron Lett.* **2001**, *42*, 4301. (b) Adam, W.; Hajra, S.; Herderich, M.; Saha-Moeller, C. R. *Org. Lett.* **2000**, *2*, 2773. (c) Wang, J.; Wei, H. X.; Schlosser, M. *Eur. J. Org. Chem.* **1999**, *3263*, 3. (d) Uenishi, J.; Motoyama, M.; Kimura, Y.; Yonemitsu, O. *Heterocycles* **1998**, *47*, 439.

(7) Review: Miller, M. J.; Vogt. P. F. *Tetrahedron* **1998**, *54*, 1317. For recent Diels−Alder reactions using (*E*,*Z*)-dienes, see: (a) Dineen, T. A.; Roush, W. R. *Org. Lett.* **2003**, *5*, 4725. (b) Waizumi, N.; Stankovic, A. R.; Rawai, V. H. *J. Am. Chem. Soc.* **2003**, *125*, 13022. (c) Roush, W. R.; Limberakis, C.; Kunz, R. K.; Barda, D. A. *Org. Lett.* **2002**, *4*, 1543.

(8) (a) Roush, W. R.; Reilly, M. l.; Koyama, K.; Brown, B. B. *J. Org. Chem.* **1997**, *62*, 8708. (b) Naruta, Y.; Nagai, N.; Yokota, T.; Maruyama, K. *Chem. Lett.* **1986**, 1185.

(9) *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; John Wiley & Sons, Ltd.: New York; 1995; Vol. 2, p 1253.

(10) (a) Shireman, B. T.; Miller, M. J.; Jonas, M.; Wiest, O. *J. Org. Chem.* **2001**, *66*, 6046. (b) Singh, A. K.; Varma, R. S. *Tetrahedron Lett.* **1992**, *33*, 2307. (c) Niwa, H.; Ito, S.; Hasegawa, T.; Wakamatsu, K.; Mori, T.; Yamada, K. *Tetrahedron Lett.* **1991**, *32*, 1329. (d) Clinch, K., Vasella, A.; Schauer, R. *Tetrahedron Lett.* **1987**, *28*, 6425. (e) Schuda, P. F.; Cichowicz, M. B.; Heimann, M. R. *Tetrahedron Lett.* **1983**, *24*, 3829. (f) Takeda, R.; Zask, A.; Nakanishi, K.; Park, M. H. *J. Am. Chem. Soc.* **1987**, *109*, 914.

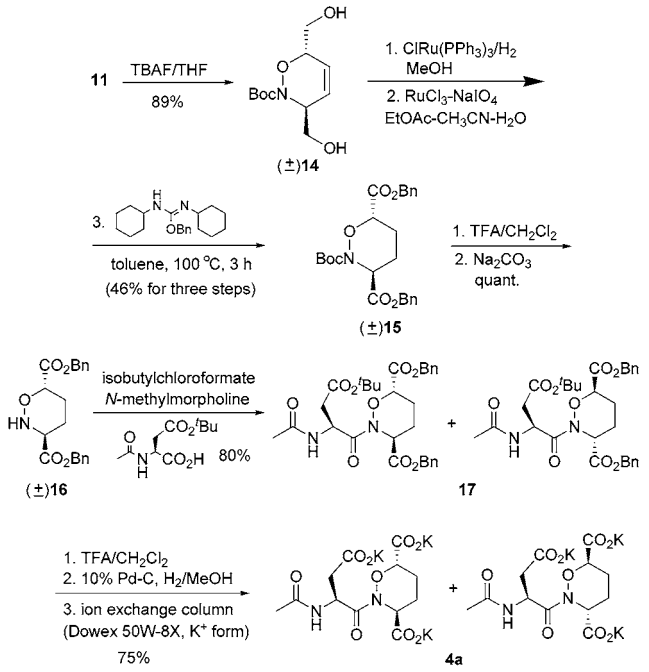
(11) (a) Review: Mathias, L. J. *Synthesis* **1979**, 561. (b) Nicolaou, K. C.; Yue, E. W.; Naniwa, Y.; Riccardis, F. D.; Nadin, A.; Leresche, J. E.; Greca, S. L.; Yang, Z. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2184. (c) Nicolaou, K. C.; Naniwa, Y.; Leresche, J. E.; Greca, S. L.; Tsuri, T.; Yue, E. D.; Yang, Z. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2187. For the preparation of the isourea reagents, see: Vowinkel, E. *Chem. Ber.* **1966**, *99*, 1479.

(12) (a) Boutin, R. H.; Rapoport, H. *J. Org. Chem.* **1986**, *51*, 5320. (b) Cupps, T. L.; Boutin, R. H.; Rapoport, H. *J. Org. Chem.* **1985**, *50*, 3972.

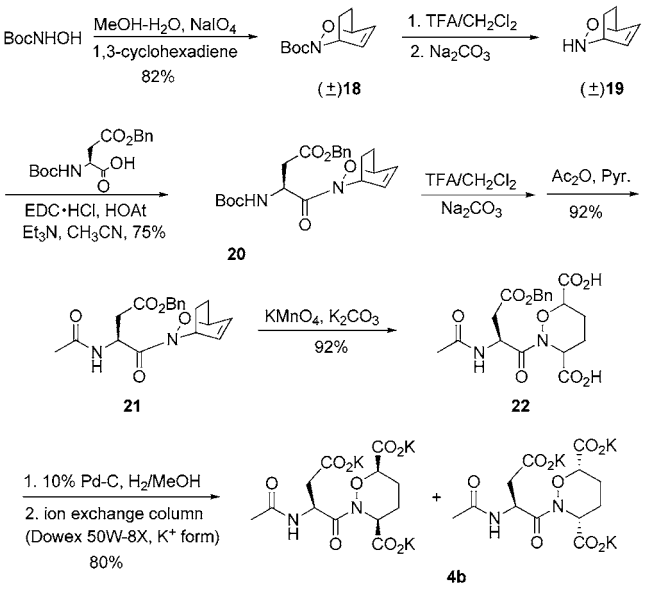
(13) Woulfe, S. R.; Miller, M. J. *Tetrahedron Lett.* **1984**, *31*, 3293.

(14) (a) Flower, K. R.; Lightfoot, A. P.; Wan, H.; Whiting, A. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2058. (b) Jenkins, N. E.; Ware, R. W., Jr.; Atkinson, R. N.; King, S. B. *Synth. Commun.* **2000**, *30*, 947. (c) Sirisoma, N. S.; Johnson, C. R. *Tetrahedron Lett.* **1998**, *39*, 2059. (d) Nelsen, S. F.; Thompson-Colon, J. A.; Kirste, B.; Rosenhouse, A.; Kaftory, M. *J. Am. Chem. Soc.* **1987**, *109*, 7128.

Scheme 5. Synthesis of Six-Membered *trans* Product **4a**



Scheme 6. Synthesis of Six-Membered *cis* Product **4b**



presence of K_2CO_3 produced diacid **22**. Hydrogenolysis of the benzyl ester of **22**, followed by ion exchange chromatography (Dowex, K^+), provided the target potassium salt **4b** in 80% yield as a mixture of diastereomers.

The activity of the PSMA inhibitors could be determined by their IC_{50} values in the NAALADase assay.[3] The ability of **4a** and **4b** to inhibit glutamate carboxypeptidase II (GCP II) was evaluated at Guilford Pharmaceuticals, Inc., using *N*-acetyl-L-aspartyl-[^{3}H]-L-glutamate as a substrate. GCP II, also known as *N*-acetyl-α-linked acidic dipeptidase (NAALADase) and prostate-specific membrane antigen (PSMA), is a metallopeptidase that cleaves *N*-acetyl-aspartyglutamate (NAAG) into *N*-acetylaspartate and glutamate.[3a] The assay results revealed that **4b** is slightly more potent (IC_{50} = 0.1 μM) than **4a** (IC_{50} = 0.9 μM). Although the biological activities of the acyclic analogues of **4** are not known, comparison with related acyclic amide substrate analogues[4] shows that conformationally restricted **4a** and **4b**, with 220 unique conformations within 3 kcal/mol of the global minimum, are respectively 25 and 220 times more active than the most closely related unrestricted analogue. On the other hand, the *cis* isomer **4b** was found, contrary to the computational prediction, to be slightly more active than *trans* isomer **4a**. The hypothesis that the most stable conformation of **VA-033** presents the carboxyl groups in an optimum position is therefore likely to be incorrect.[16]

In summary, two targeted analogues of PSMA inhibitors **4a** and **4b** have been designed and synthesized. We have demonstrated that (*E*,*Z*)-diene **10** could be utilized to carry out the nitroso Diels−Alder reaction to build the 1,4-*trans* adduct. The structures of the Diels−Alder adducts were verified by utilizing an independent route. In addition, we were able to use $RuCl_3$−$NaIO_4$ as an oxidation reagent to oxidize a diol directly to the diacid. The biological activity of the obtained compounds was found to be more potent than less conformationally unrestricted analogues.

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Supporting Information Available: NMR spectra for products **6**−**12**, **14**, **15**, **17**, **20**−**22**, **4a**, and **4b**, as well as experimental procedures and characterization data for all these compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL049473R

(15) Xu, Y.; Miller, M. J. *J. Org. Chem.* **1998**, *63*, 4314.

(16) For a very recent example where a bioactive conformation deviates from the minimal conformation of the ligand, see: Perola, E.; Charifson, P. S. *J. Med. Chem.* **2004**, ASAP (DOI: 10.1021/jm030563w).