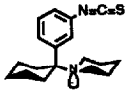
Tetrahedron Letters,Vol.30,No.35,pp 4613-4616, 1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

SYNTHESIS OF A BIOTIN CONJUGATE OF THE PCP ANALOGUE "METAPHIT" FOR POTENTIAL USE IN NMDA/PCP RECEPTOR ISOLATION

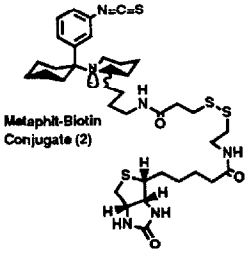
Alan P. Kozikowski and Werner Tückmantel+*
*Departments of Chemistry and Behavioral Neuroscience, Chevron Science Center, University of Pittsburgh, Pittsburgh, Pennsylvania, 15260
Karl Krueger
Fidia Georgetown Institute for the Neurosciences, 3900 Reservoir Road, N.W., Washington, D.C. 20007

SUMMARY: A high yielding synthesis of a biotinylated analogue **2** of metaphit for possible use in NMDA/PCP receptor isolation is reported. The inability of this agent to displace [^{3}H]MK-801 binding is interpreted in terms of the physical location of the PCP recognition site within the NMDA receptor-operated ion channel.

The meta-isothiocyanato derivative of phencyclidine (PCP) known as metaphit (**1**),[1] has been shown to serve as a site-directed alkylator of PCP receptors.[2] Because of the ability of metaphit to engage in stable, covalent bond-formation with PCP recognition sites,[3] we have considered its use in the preparation of a conjugate with biotin[4] for application to PCP receptor isolation. The isolation and characterization of this receptor is of paramount importance to gaining a molecular perspective on affective disorders such as schizophrenia.[2] Very recent solubilization studies, in fact, suggest that certain PCP binding sites and the NMDA subtype of glutamatergic receptors constitute a single molecular complex. In this letter we outline the synthesis of the biotin-metaphit conjugate **2**. While the chemical methods used to elaborate conjugate **2** are fairly straightforward, the general approach taken may serve as a useful guide to the preparation of other ligand-biotin conjugates for receptor isolation.[5]



Metaphit (1)



Metaphit-Biotin Conjugate (2)

Accordingly, the major product **3** (Scheme 1) formed in the bromination reaction[6] of PCP was reacted sequentially with mercuric acetate, hydrogen sulfide (to remove the mercury salts), and then with sodium cyanide at a pH of ~5.5.[7] This three step sequence proceeded in 80% overall yield to provide the α-cyanopiperidine derivative **4**. Next this nitrile **4** was reacted with 3-butenylmagnesium bromide in ether at 0 °C to furnish the phencyclidine derivative **5** containing an appropriate "arm" for linkage to biotin.

The bromine atom was now replaced by an amino group through a sequence of steps involving: (i) halogen-metal exchange using *n*-BuLi in ether and hexane; (ii) trapping of the resulting anion with tosyl azide;[8] (iii) hydrolysis of the reaction mixture with aqueous disodium ethylenediamine tetraacetate dihydrate (56% overall yield); (iv) reduction of the azide with $LiAlH_4$ in THF (93%).

Since we had planned to tether the metaphit ligand to biotin through an amide linkage by employing the commercially available biotinylating reagent NHS-SS-biotin (Pierce Chemical Co.), it became necessary to convert the olefinic arm of **7** to a primary amine. This transformation was accomplished by carrying out a

+ Current address: Pharmazeutisch-Chemisches Institut der Universität, Im Neuenheimer Feld 354, 6900 Heidelberg, West Germany.

hydroboration reaction with excess 9-BBN followed by oxidative workup to provide the alcohol **8** in 67% yield.[9] While a variety of methods were examined in turn to convert the hydroxyl group to an amine, formation of an azide by use of HN_3, PPh_3, DEAD in CH_2Cl_2 at rt (77%)[10] and subsequent hydrogenation over PtO_2 (95%) proved most efficient.

Coupling of the triamine **10**[11] with NHS-SS-biotin was found to proceed with the desired site selectivity to afford the amide **11** resulting from coupling at the less hindered, more nucleophilic, aliphatic amino group. This reaction was best carried out in an aqueous THF solution, and the reaction product purified by chromatography on silica gel using 17:2:1 *t*PrOH/H_2O/conc. NH_3 as the eluent. Lastly, the amide **11** was treated with thiophosgene in a THF/H_2O mixture containing sodium bicarbonate.[12] The desired isothiocyanate **2** was isolated in 49% overall yield as a light-amber foam after purification by preparative TLC (SiO_2, 4:1 $CHCl_3$/MeOH as the developing solvent). Compound **2** exhibited the following spectral properties: ^{1}H NMR ($CDCl_3$) δ 7.35 - 7.20 (m, 3 H), 7.09 (m, 1 H), 6.70 (br, 1 H), 6.13 (br, 1 H), 5.82 (br, 1 H), 4.97 (br, 1 H), 4.53 (dd, 1 H, *J* = 4.5 Hz, 7.5 Hz), 4.36 (dd, 1 H, *J* = 4.5 Hz, 7.5 Hz), 3.55 (q, 2 H, *J* = 6 Hz), 3.17 (m, 4 H), 3.01 (t, 2 H, *J* = 7 Hz), 2.93 (dd, 1 H, *J* = 4.5 Hz, 13 Hz), 2.86 (t, 2 H, *J* = 6 Hz), 2.75 (d, 1 H, *J* = 13 Hz), 2.63 (t, 2 H, *J* = 6.5 Hz), 2.26 (t, 2 H, *J* = 7 Hz), 2.25 - 2.05 (m, 4 H), 1.8 - 1.05 (m, 25 H), 0.85 (m, 1 H). The following first-order correlations were exhibited in a COSY spectrum: δ 4.53/4.36, 4.53/2.93, 4.36/3.17, 3.55/2.86, 3.17/~1.35, 3.01/2.63, 2.93/2.75, 2.26/1.70, 1.70/~1.45; IR (neat) 3291, 3075, 2924, 2853, 2112, 1700, 1646, 1547, 1462, 1264, 735, 698 cm^{-1}; MS (EI) m/z 425 (1%), 382 (1%), 299 (15%), 215 (18%), 148 (10%), 129 (9%), 84 (100%); Calcd for $C_{25}H_{35}N_3OS$: 425.2501; Found: 425.2500.

The scheme developed for the preparation of **2** is noteworthy for the lack of need to employ any protecting group strategies. The value of the azido group as a precursor to both aliphatic and aromatic amines is also further underscored by this study.[13] While the choice of positions on the PCP molecule for attachment of the linker arm was founded upon structure-activity information garnered either from the literature or developed by us,[14] in receptor binding assays **2** was unfortunately found inactive. This compound was tested in a concentration range of 0.1-200 μM for its ability to compete with [^{3}H]MK-801 (5 nM) binding in the presence of 5 μM glutamate. MK-801, a structurally rigid molecule, binds with high affinity to the PCP recognition site which is located within the NMDA receptor-associated ion channel.[15] These membrane binding assays were performed using bovine cerebral cortex synaptosomal plasma membranes, and the nonspecific binding was determined in parallel samples containing 10 μM nonradioactive MK-801.[16] Nonspecific binding was found to be less than ten percent of the total binding to the membranes.

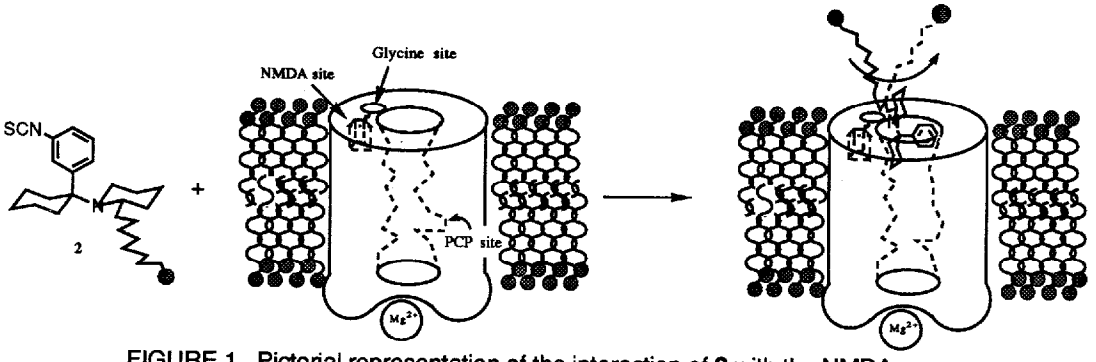
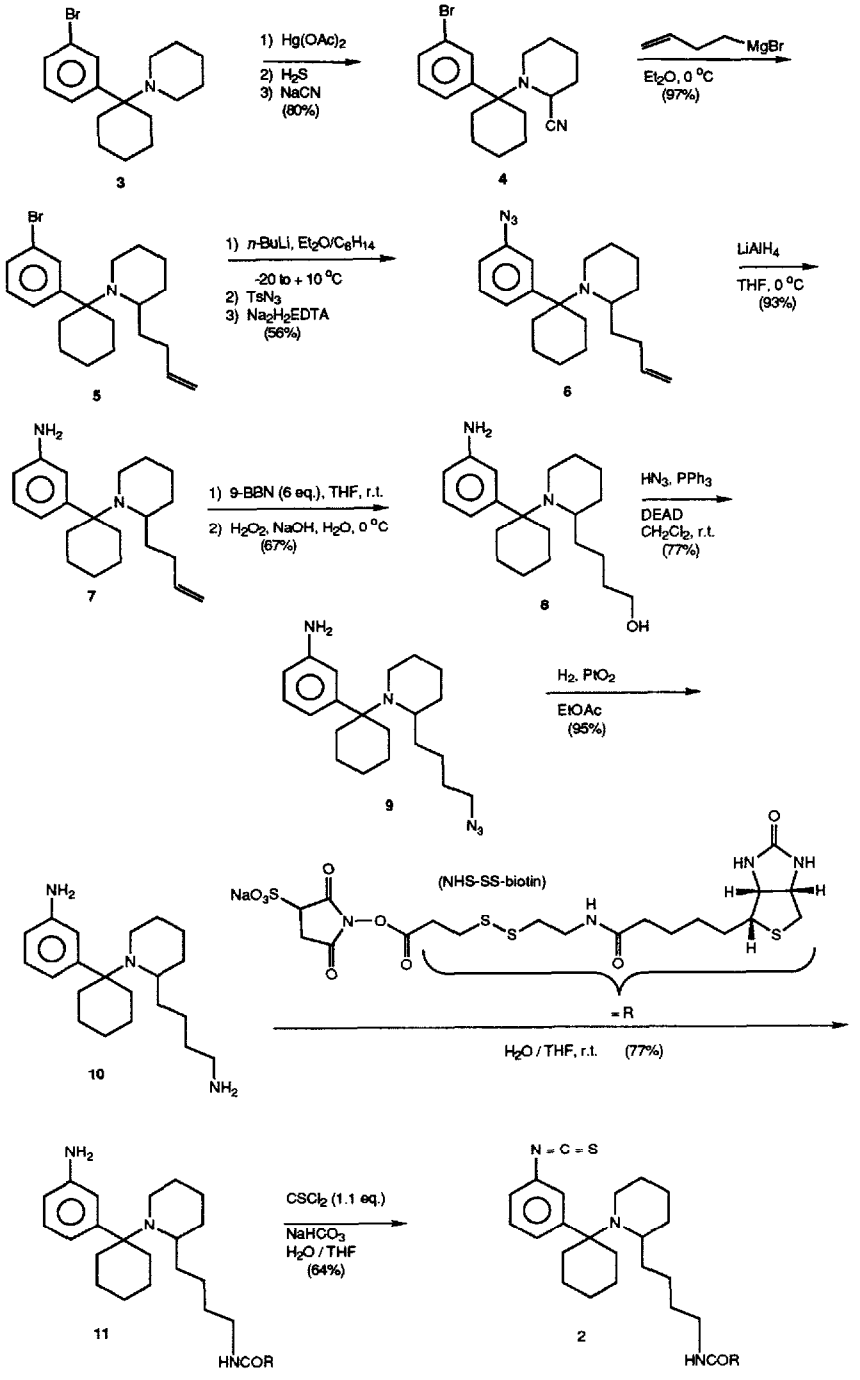


FIGURE 1. Pictorial representation of the interaction of **2** with the NMDA receptor-operated ion channel (structures not to scale).

SCHEME 1. Synthesis of a Metaphit-Biotin Conjugate



It is currently our belief that the failure of **2** to displace MK-801 binding is a consequence of the conformational bulk of its linker arm which impedes the threading of the metaphit moiety into the approximate center of the NMDA receptor-associated ion channel (see Figure 1).[17] Additionally, the site chosen for attachment of the linker arm on metaphit may be inappropriate for attainment of the required binding conformation.[18]

Acknowledgement

We are indebted to the Fidia Research Foundation, Washington, D.C. for its support of these studies.

References

1. M. F. Rafferty, M. Mattson, A. E. Jacobson, and K. C. Rice, *Febs. Lett.*, **181**, 318-322 (1985).
2. P. C. Contreras, S. Johnson, R. Freedman, B. Hoffer, K. Olsen, M. F. Rafferty, R. A. Lessor, K. C. Rice, A. E. Jacobson, and T. L. O'Donohue, *J. Pharmacol. Exp. Ther.*, **238**, 1101-1109 (1986); C. A. Tamminga, K. Tanimoto, S. Kuo, T. N. Chase, P. C. Contreras, K. C. Rice, A. E. Jackson, and T. L. O'Donohue, *Synapse*, **1**, 497-504 (1987).
3. J. P. Vincent, B. Kartalovski, P. Geneste, J. M. Kamenka, and M. Lazdunski, *Proc. Natl. Acad. Sci., USA*, **76**, 4678-4682 (1979).
4. K. Hofmann, G. Titus, J. A. Montibeller, and F. M. Finn, *Biochem.*, **21**, 978-984 (1982); M. Shimkus, J. Levy, and T. Herman, *Proc. Natl. Acad. Sci., USA*, **82**, 2593-2597 (1985).
5. K. Hofmann and F. M. Finn, *Ann. N.Y. Acad. Sci.*, **447**, 359-372 (1985).
6. K. E. MaloneyHuss, Ph.D. Dissertation, University of Pittsburgh, 1987.
7. N. J. Leonard and A. G. Cook, *J. Am. Chem. Soc.*, **81**, 5627-5631 (1959).
8. P. A. S. Smith, C. D. Rowe, and L. B. Bruner, *J. Org. Chem.*, **34**, 3430-3433 (1969).
9. E. F. Knights and H. C. Brown, *J. Am. Chem. Soc.*, **90**, 5280-5281 (1968).
10. H. Loibner and E. Zbiral, *Helv. Chim. Acta.*, **59**, 2100-2113 (1976).
11. Azide **9**: ^{1}H NMR ($CDCl_3$) δ 7.07 (t, 1 H, J = 8 Hz), 6.77 (d, 1 H, J = 8 Hz), 6.70 (t, 1 H, J = 2 Hz), 6.54 (dd, 1 H, J = 2 Hz, 8 Hz), 3.59 (br s, 2 H), 3.12 (t, 2 H, J = 7 Hz), 3.00 - 2.88 (m, 2 H), 2.50 (m, 1 H), 2.24 - 2.03 (m, 2 H), 1.91 (m, 1 H), 1.8 - 1.0 (m, 18 H), 0.70 (m, 1 H); IR (neat) 3459, 3370, 2930, 2855, 2095, 1617, 1603, 1493, 1453, 1256, 781, 706 cm^{-1}; MS(EI) m/z 355 (5%, M^+), 312 (3%), 257 (21%), 174 (29%), 173 (18%), 106 (24%), 84 (100%); Calcd for $C_{21}H_{33}N_5$: 355.2736; Found: 355.2737.
    Triamine **10**: ^{1}H NMR ($CDCl_3$) δ 7.07 (t, 1 H, J = 8 Hz), 6.78 (d, 1 H, J = 8 Hz), 6.71 (s, 1 H), 6.54 (dd, 1 H, J = 2 Hz, 7.5 Hz), 3.00 - 2.88 (m, 2 H), 2.56 (t, 2 H, J = 7 Hz), 2.52 (m, 1 H), 2.25 - 0.95 (m, 21 H), 0.73 (m, 1 H); IR (neat) 3339, 3212, 2928, 2851, 1603, 1453, 1308, 1156, 1076, 777, 706 cm^{-1}; MS (EI) m/z 329 (16%, M^+), 286 (18%), 257 (100%), 174 (100%), 173 (48%), 155 (60%), 106 (42%), 84 (83%); Calcd for $C_{21}H_{25}N_3$: 329.2831; Found: 329.2833.
12. M. Uher and J. Jendrichovsky, *Coll. Czech. Chem. Commun.*, **38**, 289-393 (1973).
13. *The Chemistry of the Azido Group*, S. Patai, Ed., Wiley Interscience, New York, 1971.
14. J. T. Wroblewski, F. Nicoletti, E. Fadda, A. P. Kozikowski, J. W. Lazarewicz, and E. Costa, in *Allosteric Modulation of Amino Acid Receptors: Therapeutic Implications*, E. A. Barnard and E. Costa, Eds., Raven Press Ltd., New York, 1989, pp. 287-299.
15. C. W. Cotman and L. L. Iverson, *Trends Neurosci.*, **10**, 263 (1987).
16. E. Fadda, W. Danysz, J. T. Wroblewski, and E. Costa, *Neuropharmacology*, in press.
17. The location of the PCP binding site within the NMDA receptor channel is based upon electrophysiological experiments which reveal PCP's action to be dependent on the voltage across the neuronal membrane. See: C. R. Honey, Z. Miljkovic, and J. F. MacDonald, *Neurosci. Lett.*, **61**, 135 (1985); J. E. Huettner and B. P. Bean, *Proc. Natl. Acad. Sci., USA*, **85**, 1307 (1988).
18. A. P. Kozikowski and Y.-P. Pang, unpublished data.

(Received in USA 22 June 1988; accepted 10 July 1989)