Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIX, No.3

SYNTHESIS OF ^{14}C-LABELLED (R)-α-AMINO-6,7-DIMETHYL-3-(PHOSPHONOMETHYL)-2-QUINOLINEPROPANOIC ACID

Britt-Marie Swahn[a], Fredrik Andersson[b], Benjamin Pelcman[a], Johan Söderberg[a] and Alf Claesson[a]*.

[a]Preclinical Research, Astra Pain Control AB, S-15185 Södertälje, Sweden. [b]Preclinical Research, Astra Arcus AB, S-15185 Södertälje, Sweden.

SUMMARY

The NMDA antagonist (R)-α-amino-6,7-dimethyl-3-(phosphonomethyl)-quinolinepropanoic acid **1** was [^{14}C]-labelled at the 6-methyl group. The quinoline synthesis started from 4-bromo-3-methylaniline and the radiolabel was introduced late in the synthetic scheme. The synthesis was accomplished by a $NiCl_2(dppp)_2$-catalyzed coupling of the 6-bromoquinoline derivative **6** with a zinc reagent made from [^{14}C]-methyl iodide.

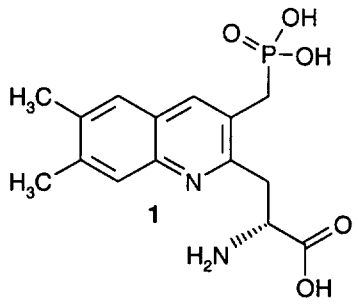
KEY WORDS: NMDA antagonist, zinc reagent, [^{14}C]-methyl iodide, $NiCl_2(dppp)_2$, nickel catalysis

INTRODUCTION

The N-methyl-D-aspartate (NMDA) receptor is a multiprotein complex that forms a ligand-gated cation channel. Antagonists of the NMDA receptor are of interest for their potential use as therapeutic agents in the treatment of epilepsy, in pain relief, and for protection against neurodegeneration associated with stroke.[1] Several selective competitive antagonists for the NMDA receptor have been described and the most potent of these share the structural features

CCC 0362–4803/97/030259-08$17.50

of an α-amino acid linked to an ω-phosphono acid moiety.[1] In order to evaluate a series of 2,3-quinoline-spaced ω-phosphono α-amino acids[2] in autoradiographic and pharmacodynamic studies, we chose to radiolabel compound **1**.



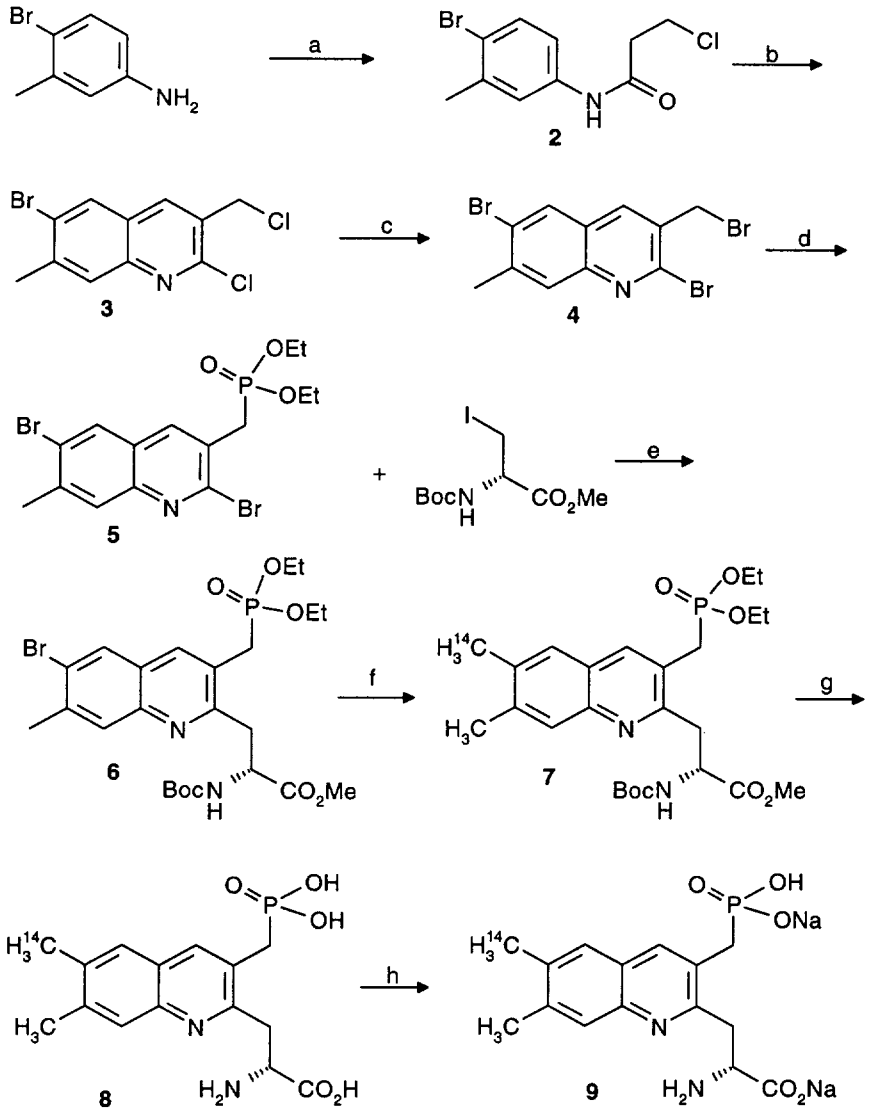
1

Naturally, we wished to use the synthetic sequence we developed for compound **1** in which the chiral amino acid is introduced late in the synthesis.[2] However, the unavailability of labelled (R)-amino acids forced us to take a different approach. Instead we envisioned the possibility of introducing one of the methyl groups late in the synthetic scheme, via a coupling reaction with an organometallic reagent made from [^{14}C]-methyl iodide.

RESULTS AND DISCUSSION

Our synthetic approach relied upon the difference in reactivity of the three different bromines in the quinoline **4**. Compound **4** was synthesized starting from commercially available 4-bromo-3-methylaniline, as shown in Scheme 1. The aniline was acylated with 3-chloropropionyl chloride affording amide **2**, which was converted to quinoline **3** in one pot by formylation with Vilsmeier reagent ($POCl_3$/DMF) and subsequent heating.[3] Four equivalents of phosphoroxy tribromide ($POBr_3$) were heated with **3** to give the pivotal intermediate **4** in high yield.

Scheme 1.



a) 3-chloropropionyl chloride, toluene (95%) b) $POCl_3$, DMF (18%) c) $POBr_3$ (80%) d) $P(OEt)_3$, toluene (87%) e) Zn-Cu, toluene, DMA, $Pd(OAc)_2$, tri-2-furylphosphine, toluene (76%) f) [^{14}C]-methyl iodide, Zn-Cu, THF, $NiCl_2(dppp)_2$ (37%) g) 6M HCl h) 1M NaOH (56% from **7**)

Decreasing the amount of $POBr_3$ invariably afforded incomplete exchange of the 2-substituent. Compound **4** was converted to the phosphonate ester **5** with triethyl phosphite under Arbuzov reaction conditions.

The most active competitive NMDA-antagonists are (R)-α-amino acids,[1,2] and by subjecting the 2-bromoquinoline **5** to a palladium-catalyzed reaction with the zinc reagent prepared from protected β-iodo-(R)-alanine,[4] it was possible to make the enantiomerically pure compound **6**. The best yields in this coupling reaction were obtained using a system comprising palladium diacetate and tri-2-furylphosphine.[2] Due to reactivity differences, only trace amounts of the coupling product in which the coupling reaction had occured in the 6-position of the quinoline ring could be detected. After separation from starting material using semipreparative reversed phase HPLC, intermediate **6** was obtained sufficiently pure for the radiolabelling step.

Although we were able to make a zinc reagent from methyliodide, our attempts to couple this with the 6-bromo substituent in **6**, using palladium as a catalyst, met with only limited success. However, a nickel catalyst, $NiCl_2(dppp)_2$, was found to be highly effective. With 4 equivalents of the zinc reagent made from methyl iodide it was possible to completely replace the 6-bromo substituent. In the radiolabelling step Zn-Cu and [^{14}C]-methyl iodide were reacted using ultrasonic activation in THF. The zinc reagent formed was used in the $NiCl_2(dppp)_2$-catalyzed coupling with intermediate **6**. The product **7** was deprotected in refluxing 6M HCl affording the amino acid **8**, which was precipitated as the disodium salt **9**. The enantiomeric purity of **9** was 95.5% as determined by HPLC analysis on a Crownpack CR(-) column,[2] and the radiochemical purity was >97% as determined by TLC.

EXPERIMENTAL

All chemicals and reagents were used as received from the suppliers, unless otherwise stated. Zinc-copper couple was made according to an Organic Synthesis procedure.[5] Methyl (R)-α-t-butoxycarbonylamino-β-iodopropionate was synthesized essentially as described by Jackson et al.[4] [^{14}C]-Methyl iodide (108 mCi, 52,9 mCi/mmol) was purchased from Zeneca Bio Products, U.K. NMR spectra were recorded on a Varian Unity 400 (400 MHz) spectrometer in $CDCl_3$; data are reported in parts per million (δ) and are referenced to $CHCl_3$ at δ7.26 for ^{1}H spectra.

Semipreparative HPLC purification was performed by using two Gilson pumps and a Gilson mixer, together with a Rainin Dynamax absorbance detector Model UV-1. The elemental microanalyses were performed at Mikro Kemi AB in Uppsala, Sweden. Radioactivity measurements were carried out with a Packard Tri-carb 1000 TR liquid scintillation spectrometer using Packard Ultima Gold as the scintillation medium. Analytical TLC was performed using silica gel 60 F-254 (E. Merck). The radioactivity measurements were carried out with a Bioscan System 2000 Imaging Scanner. All labelled compounds were compared with the unlabelled reference compounds by TLC or HPLC.

3-Chloro-N-(4-bromo-3-methylphenyl)propionamide (2).

3-Chloropropionyl chloride (10.9 mL, 114 mmol), dissolved in 20 mL of toluene, was added at 0°C to a stirred solution of 4-bromo-3-methylaniline (21.2 g, 114 mmol) and triethylamine (15.9 mL, 114 mmol) in 200 mL of toluene. After stirring for 2 hours at room temperature water (100 mL) was added. The resulting mixture was extracted with ethyl acetate. The organic phase was dried over $MgSO_4$, filtered through a silica pad, and then concentrated *in vacuo* to give 30 g (95%) of **2** as a solid. ^{1}H NMR δ 2.29 (s, 3H, $-CH_3$), 2.78 (t, 2H,$-CH_2-$), 3.83 (t, 2H,$-CH_2Cl$), 7.17 (dd, 1H, ArH), 7.39 (m, 2H, ArH).

6-Bromo-2-chloro-3-chloromethyl-7-methylquinoline (3).

Phosphorus oxychloride (51 mL, 545 mmol) was stirred and cooled in an ice bath, then dry dimethyl formamide (9 mL, 116 mmol) was added at such a rate as to maintain the temperature at 0-5°C. After the addition was completed the mixture was stirred for 5 minutes before **2** (21.5 g, 78 mmol) was added in one portion. The mixture was stirred and heated at 80°C for 10 hours. The cooled reaction solution was poured onto crushed ice and the resulting mixture was extracted with methylene chloride. The organic phase was washed with saturated sodium hydrogen carbonate solution, dried over $MgSO_4$ and concentrated *in vacuo*. Flash chromatographic purification over silica gel with toluene as eluent afforded 4.2 g (18%) of **3** as

a solid. ^{1}H NMR δ 2.58 (s, 3H, -CH_3), 4.79 (s, 2H, -CH_2Cl), 7.84 (s,1H, ArH), 8.01 (s, 1H, ArH), 8.12 (s, 1H, ArH). Anal. Calcd for $C_{11}H_8BrCl_2N$: C, 43.3; H, 2.6; N, 4.6. Found: C, 43.8; H, 2.7; N, 4.4.

3-Bromomethyl-2,6-dibromo-7-methylquinoline (4).

Phosphorus oxybromide (15.7 g, 55 mmol) was added to **3** (4.18 g, 14 mmol) and the mixture was heated at 100°C for 4h. The solution was allowed to cool before being poured onto crushed ice. The resulting mixture was extracted with methylene chloride and the organic phase was washed with saturated sodium hydrogen carbonate solution, dried over $MgSO_4$ and concentrated *in vacuo* to yield 4.3 g (80%) of **4** sufficiently pure to be used in the next step without further purification. An analytical sample was recrystallized from toluene. ^{1}H NMR δ 2.58 (s, 3H,-CH_3), 4.69 (s, 2H, -CH_2Br), 7.86 (s, 1H, ArH), 7.99 (s, 1H, ArH), 8.06 (s,1H, ArH). Anal. Calcd for $C_{11}H_8Br_3N$: C, 33.5; H, 2.0; N, 3.6. Found: C, 34.2; H, 2.0; N, 3.5.

2,6-Dibromo-3-diethylphosphonomethyl-7-methylquinoline (5).

A mixture of **4** (3.0 g, 7.6 mmol), toluene (50 mL) and triethylphosphite (2.7 mL, 15.5 mmol) was heated at reflux for 10 hours. After cooling, the solution was concentrated *in vacuo* and the residue was purified by chromatography on silica gel with ethyl acetate/ toluene (2:1) as eluent. The product precipitated upon addition of hexane. The crystals were filtered off, washed with hexane and dried *in vacuo* to give 3.0 g (87%) of **5**. ^{1}H NMR δ 1.28 (t, 6H,-CH_2CH_3), 2.59 (s, 3H,-CH_3), 3.52 (d, 2H,-CH_2P), 4.10 (q, 4H,-CH_2CH_3), 7.87 (s, 1H, ArH), 8.01 (s, 1H, ArH), 8.11 (d, 1H, ArH). Anal. Calcd for $C_{15}H_{18}Br_2NO_3P$: C, 39.9; H, 4.0; N, 3.1; P, 6.9. Found: C, 40.3; H, 4.0; N, 3.1; P, 6.9.

Methyl (R)-α-t-butoxycarbonylamino-6-bromo-3-diethylphosphonomethyl-7-methyl-2-quinolinepropionate (6).

A solution of methyl (R)-α-t-butoxycarbonylamino-β-iodopropionate (4.38 g, 13.3 mmol) in 80 mL of dry toluene and 8 mL of dry dimethylacetamide was added to a nitrogen purged flask charged with zinc-copper couple (1.73 g, 26.6 mmol). The mixture was treated in an ultrasonic bath for 1 hour at 45°C until no starting material remained, as judged by TLC (toluene/ ethyl

acetate 9:1). A mixture of $Pd(OAc)_2$ (0.15 g, 0.67 mmol) and tri-2-furylphosphine (0.31 g, 1.34 mmol) was added followed by **5** (3.0 g, 6.65 mmol). The reaction mixture was stirred for 4 hours at 50-60°C. After cooling the reaction mixture was diluted with ethyl acetate, washed with saturated sodium hydrogen carbonate solution, dried over $MgSO_4$ and concentrated. The crude product was subjected to chromatography on silica gel with ethyl acetate as eluent to yield 2.9 g (76%) of **6**. This material had to be separated from small amounts of starting material by HPLC on a semipreparative column, Dynamax-60A, RP C-18, 21.4mm x 250mm, CH_3CN/0.06M phosphate buffer (60/40), pH=4, 24mL/min, UV detection at 252nm. ^1H NMR δ 1.25 (t, 6H,-CH_2CH_3), 1.41, (s, 9H, BOC), 2.58 (s, 3H,-CH_3), 3.28 (d, 2H,-CH_2-P), 3.28 (m, 1H, Ar-CH_2-), 3.49 (s, 3H, OCH_3), 3.73 (m, 1H, Ar-CH_2-), 4.05 (q, 4H, -CH_2CH_3), 4.90 (m, 1H, -CH-), 6.03 (d, 1H, -NHBOC), 7.80 (s, 1H, ArH), 7.95 (s, 1H, ArH) 7.96 (s, 1H, ArH). Anal. Calcd for $C_{24}H_{34}BrN_2O_7P$: C, 50.3; H, 6.0; N, 4.9; P, 5.4. Found: C, 49.5; H, 5.9; N, 4.8; P, 5.4.

Methyl (R)-α-t-butoxycarbonylamino-3-diethylphosphonomethyl-[6-^{14}C]-7-dimethyl-2-quinolinepropionate (7).

[^{14}C]-Methyl iodide (108 mCi, 2.0 mmol) dissolved in THF (2 mL) was added to Zn-Cu (183 mg, 2.8 mmol) and the mixture was treated in an ultrasonic bath for 2 hours at 30°C. [1,3-Bis(diphenylphosphino)propane]nickel(II) chloride (55 mg, 0.1 mmol) was added followed by a solution of **6** (294 mg, 0.51 mmol) in THF (2 mL). The reaction mixture was then stirred at 60°C for 16 hours. The reaction mixture was concentrated using a flow of N_2 gas. The residue was diluted with EtOAc (10 mL) and extracted with H_2O (5 mL). The water phase was extracted twice with EtOAc (2 x 3 mL) and the combined organic phases were washed with brine (3 mL), dried over $NaSO_4$ and concentrated to yield 24 mCi of crude **7**. Chromatography on silica gel with EtOAc as eluent gave **7** (9.9 mCi, 37 % yield). The radiochemical purity was >99 % as determined by TLC analysis (R_f= 0.39, EtOAc).

Analytical data for the unlabelled compound **7**. ^1H NMR δ 1.18 (t, 6H,-CH_2CH_3), 1.34, (s, 9H, BOC), 2.34 (s, 3H,-CH_3), 2.37 (s, 3H,-CH_3), 3.20 (d, 2H,-CH_2-P), 3.42 (m, 1H, Ar-CH_2-),

3.61 (s, 3H, OCH_3), 3.67 (m, 1H, Ar-CH_2-), 3.97 (q, 4H, -CH_2CH_3), 4.82 (m, 1H, -CH-), 6.08 (d, 1H, -NHBOC), 7.40 (s, 1H, ArH), 7.63 (s, 1H, ArH) 7.87 (d, 1H, ArH).

(R)-α-amino-[6-^{14}C]-7-dimethyl-3-phosphonomethyl-2-quinolinepropanoic acid (8).

A cooled 6M HCl (2.5 mL) solution was added to **7** (0.19 mmol) and the mixture was stirred for 3 hours at 0°C and then at 95°C for 8 hours. The resultant solution was concentrated using a flow of N_2 gas and used directly in the next step.

(R)-α-amino-[6-^{14}C]-7-dimethyl-3-phosphonomethyl-2-quinolinepropanoic acid disodium salt (9).

The residue **8** was dissolved in H_2O (2 mL) and pH was adjusted to 3.2 with 1M NaOH. The resultant suspension was centrifuged (4000 v/min, 10 min) and the supernatant was removed. H_2O (0.5 mL) was added to the solid and the pH was adjusted to 8.4 with 1M NaOH. Addition of acetone precipitated the disodium salt **9**. The mixture was centrifuged (4000 v/min, 10 min) and the supernatant was removed. The residue was dissolved in H_2O (2 mL) to give 5,5 mCi of **9** (56 % yield from **7**). The radiochemical purity determined by TLC analysis (R_f=0.31, BuOH-HOAc-H_2O, 3/2/2) was 97.6%.

Analytical data for the unlabelled compound **9**. ^{1}H NMR(D_2O): δ 2.23 (s, 3H,-CH_3), 2.26 (s, 3H, CH_3) 2.80 (m, 2H,-CH_2-P), 3.35 (m, 2H, Ar-CH_2-), 4.12 (m, 1H, -CH-), 7.36 (s, 1H, Ar5H), 7.46 (s, 1H, Ar8H) 7.76 (d, 1H, Ar4H). Anal. Calcd for $C_{15}H_{19}N_2O_5P$*2Na*H_2O: C, 44.7; H, 5.3; N, 6.9; P, 7.7. Found: C, 44.4; H, 5.2; N, 6.9; P, 7.5.

REFERENCES

1. Meldrum B.S. - Excitatory Amino Acid Antagonists, Blackwell Sci. Publ. (1991)
   Bigge C. F. - Biochem. Pharmacol. 45: 1547 (1993)
   Ornstein P. L., Monn J. A., Schoepp D. D. - Drug News Perspec. 7: 5 (1994)
2. Swahn B-M., Claesson A., Pelcman B., Besidski Y., Molin H., Sandberg M. P., Berge O.-G. - Bioorg. Med. Chem. Lett. 6: 1635 (1996)
3. Meth-Cohn O., Rhouati S., Tannowski B., Robinson A. - J. Chem. Soc., Perkin Trans. I. 1537 (1981)
4. Jackson R. F. W., Wishart N., Wood A., James K., Wythes M. J. - J. Org. Chem. 57: 3397 (1992)
5. Smith R. D., Simmons H. E. - Org. Synth. Coll. Vol. V. 855 (1973)