PROBING THE TCDD RECEPTOR: SYNTHESIS *of* 5,6-DIHYDRO-6a,11,11b-TRIAZABENZO[*a*]FLUORENE · PF_6, A POTENTIAL LIGAND

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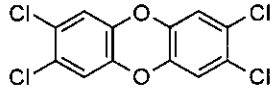
Abstract - With the aim of investigating whether the ability of omeprazole (**3**) to activate the TCDD receptor is due to an internal cyclization process, 5,6-dihydro-6a,11,11b-triazabenzo[*a*]fluorene · PF_6 (**6**) (X=PF_6) has been designed and synthesized *via* a one-pot Mitsunobu reaction. An improved synthesis of the tosylate (**10**) of 2-(2-hydroxyethyl)pyridine has also been developed.

INTRODUCTION

The TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin (**1**)) receptor is a ubiquitous, intracellular protein present in virtually all rodent tissues or human cells examined.[1] Another name for the same receptor commonly used in the literature is the aryl hydrogen (Ah) receptor protein. Upon binding of a proper ligand the resulting receptor-ligand complex is translocated to the nucleus where it will act as transcription factor for several genes. These genes encode proteins involved in the metabolism of xenobiotica as well as in cell growth and differentiation. Most notably, cytochrome P-4501A1 (CYP1A1), considered to play a major role in the activation of procarcinogens, is induced by TCDD-like substances.[1a,b,2] The hitherto known ligands of the receptor include polychlorinated aromatic hydrocarbons, polycyclic aromatic hydrocarbons, and some compounds of dietary origin, e.g. indolo[3,2-*b*]carbazole (**2**).[3]



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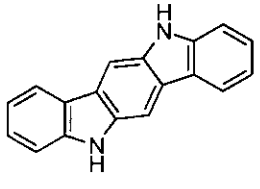

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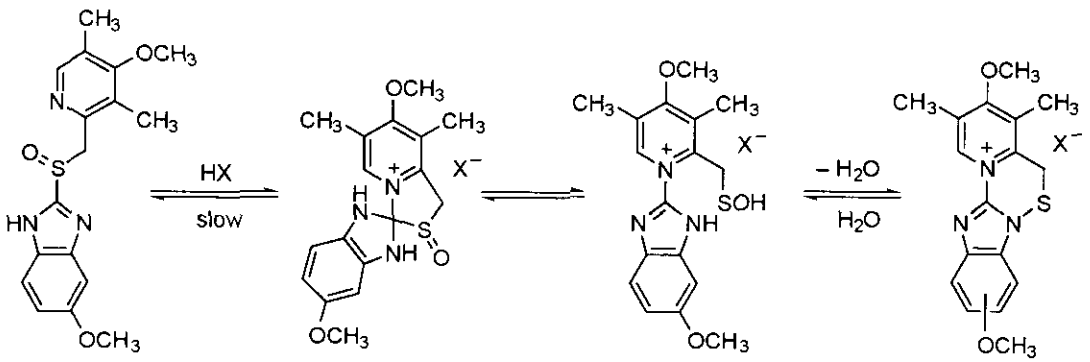
The importance of the signal pathways involving the TCDD receptor in mammals, including man, can hardly be overestimated. Recent data appear to favor a physiological role for the TCDD receptor in addition to its function in xenobiotic metabolism:[4] TCDD receptor deficient mice have a low survival rate

and show impaired development of the liver and the immune system.[4] A physiological ligand of the receptor has not yet been identified.

Recently, it has been demonstrated that omeprazole (Losec®) (**3**), the leading anti-ulcer drug, activates the TCDD receptor into a DNA binding form *in vivo*, but not *in vitro*.[5] This might seem surprising since omeprazole does not show many structural similarities with the planar ligands mentioned above. However, studies of the metabolism and action of omeprazole[6,7] have shown that it proceeds *via* a cyclic sulfenamide (**4**), which by virtue of its structure would seem to be a possible candidate for TCDD receptor binding (Scheme 1).

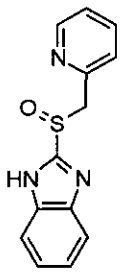


3 **4**

Scheme 1

This transformation might also explain the fact that omeprazole induces DNA binding of the receptor *in vivo*, but not *in vitro* : enzymes in the cells may be needed to convert the sulfoxide (**3**) into the tetracyclic compound (**4**).

Omeprazole exerts its anti-ulcer action through the formed sulfenamide (**4**): thiol groups of the gastric (H^+-K^+)ATPase attack the sulfenamide leading to covalent modification of the enzyme and thereby inhibition of gastric acid secretion. The existence of **4** was first inferred from indirect evidence due to its instability, but subsequently it has been possible to isolate **4** and variously substituted analogues as their tetrafluoroborate salts under, what it seems, very well-controlled conditions.[8] Uncertainty about the stability of sulfenamides in our cell systems induced us to initiate our study of the cyclization hypothesis with a somewhat simpler system, namely the omeprazole analog (**5**) and the more robust sulfenamide analog (**6**). Considering the known ligands of the TCDD receptor mentioned above, the sulfur atom of the sulfenamide should be of minor concern regarding *affinity* to the receptor (addition of any thiol groups of the receptor is another matter). Furthermore, it would also give us an opportunity to synthesize a type of ring system that, to the best of our knowledge, has never been encountered before. It is worth mentioning that an attempt to isolate the unsubstituted sulfenamide (**7**) (X=BF_4) failed due to its instability.[8]



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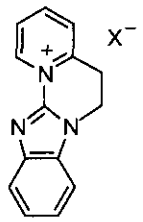

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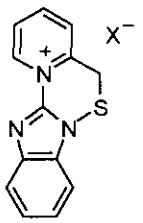

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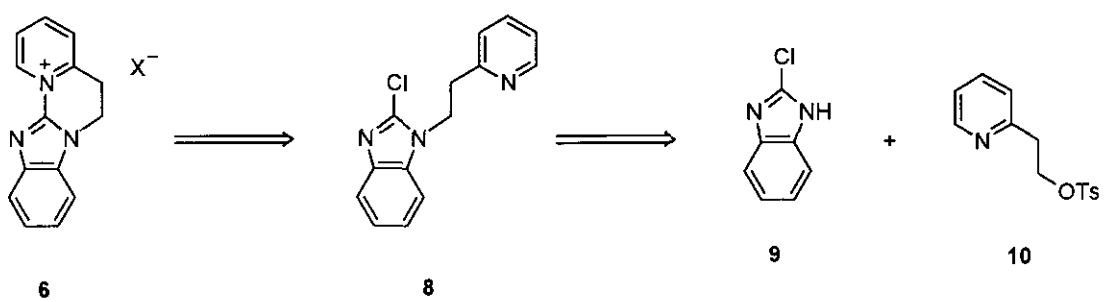
In control experiments the known compound (**5**)[9] showed to behave similarly to omeprazole (**3**) in our chosen cell systems,[5] thus indicating that the nature of the ring substituents were not so important in this context and thereby justifying the use of compounds (**5**) and (**6**) as models. We therefore proceeded to synthesize compound (**6**).

RESULTS AND DISCUSSION

A retrosynthetic analysis is outlined in Scheme 2. Since it is well known that pyridine reacts with 2-chlorobenzimidazole (**9**) at the 2-position to give a pyridinium ylide[10,11] (heating 1-2 days, 36 %), the cyclization of compound (**8**) seemed to be a promising final step. This precursor (**8**) could potentially be produced by coupling of the known 2-chlorobenzimidazole[12] (**9**) with the tosylate (**10**)[13] of 2-(2-hydroxyethyl)pyridine.

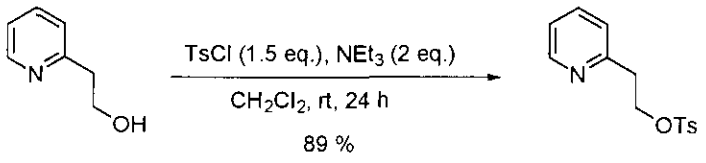


6 8 9 10

Scheme 2

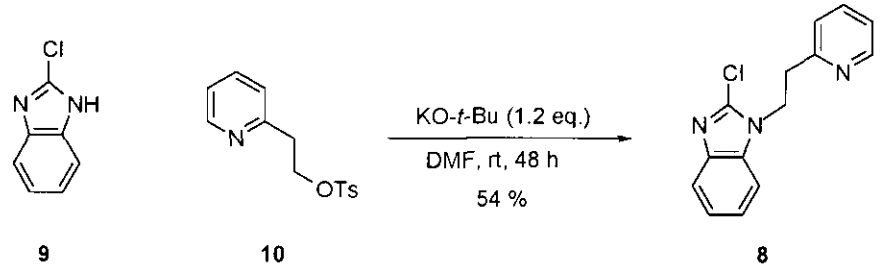
Since the information concerning the synthesis of the tosylate (**10**)[13] was very scarce and the yield moderate (60 %), we decided to develop a new method. With a slight modification of the protocol of

Kabalka[14] 2-(2-hydroxyethyl)pyridine (**11**) was tosylated using triethylamine as base (instead of pyridine) in 89 % yield (Scheme 3).



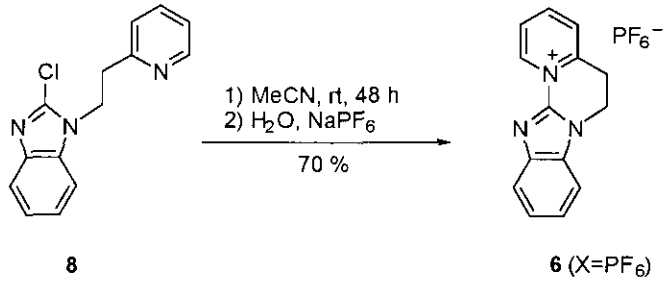
Scheme 3

2-Chlorobenzimidazole (**9**)[12] was then coupled in the presence of potassium *tert*-butoxide with the tosylate (**10**) in DMF at room temperature to give **8** (a sensitive molecule) in 54 % yield after 48 h (Scheme 4).



Scheme 4

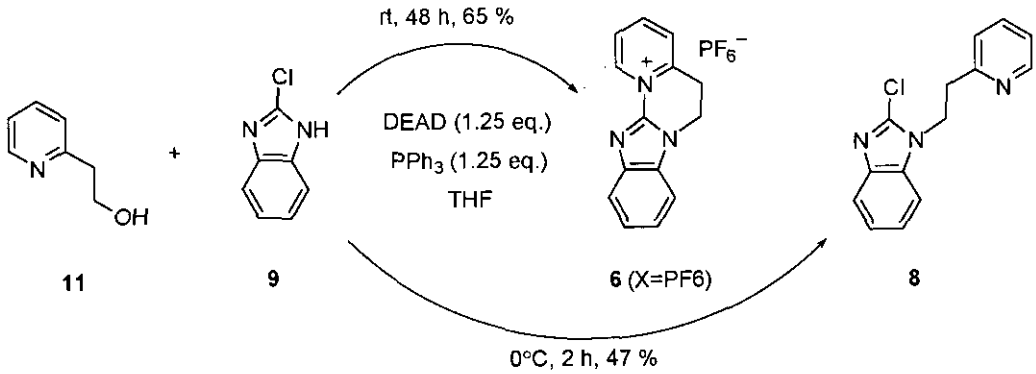
Utilization of sodium hydride as base resulted in a slower reaction, and raising the temperature (up to 50°C) gave a less clean reaction (including the formation of 2-vinylpyridine).
The cyclization of compound (**8**) was preferably performed in MeCN at room temperature and the tetracycle (**6**) was isolated as its hexafluorophosphate salt (Scheme 5).



Scheme 5

Although slow, raising the temperature was not well-advised since it resulted in a less clean product. The formation of **6** was probably catalyzed by trace amounts of acid: solutions of **8** in $CDCl_3$ (known to contain traces of DCl) changed color within hours and **6** (X=Cl) was gradually formed. In contrast, **8** seemed to be infinitely stable in solutions of DMSO. Since **8** in neat form was not long-lived even in a freezer, characterization took place immediately after purification.

The sluggishness and the moderate yield of the reaction between 2-chlorobenzimidazole (**9**)[12] and tosylate (**10**)[13] prompted us to try the Mitsunobu reaction[15] with 2-(2-hydroxyethyl)pyridine (**11**) and 2-chlorobenzimidazole (**9**)[12] as reaction partners (Scheme 6). To our joy, the coupling did not only take place at room temperature, but the intermediate (**8**) cyclized spontaneously to give the desired tetracycle (**6**) (X=Cl), which could once again be isolated as its hexafluorophosphate salt (**6**) ($X=PF_6$) in 65 % yield!



Scheme 6

As a matter of fact, it was difficult to isolate the intermediate (**8**) using this method. Running the reaction at 0°C prevented the cyclization and the consumption of starting material was complete within 2 h. The well-known problem[15] of getting rid of the formed triphenylphosphine oxide was experienced also in this case: isolation of **8** could only be achieved *via* an extractive procedure with dilute hydrochloric acid. Since **8** is acid-sensitive, this most probably meant loss of material. The final result was a meagre 47 % yield after chromatography.

CONCLUSION

In summary, we have synthesized the tetracyclic substance (**6**), intended to be used as a model for sulfenamide (**4**) in biological experiments aiming at explaining the *in vivo*, but not *in vitro* induction of the TCDD receptor by omeprazole (**3**). Biological experiments are currently in progress.

EXPERIMENTAL SECTION

With the following exceptions all reagents and solvents were purchased from commercial suppliers and used without further purification: 2-(2-hydroxyethyl)pyridine (**11**) was kindly donated by Reilly and distilled before use; 2-chlorobenzimidazole (**9**) was synthesized according to Smith;[12] DMF was distilled from CaH_2; THF was distilled from Na/benzophenone, and distilled solvents were used for flash chromatography. The petroleum ether used for chromatography had the boiling point range 60-80°C. Silica gel (230-400 mesh) for column chromatography as well as corresponding TLC plates were purchased from Merck. Experiments involving dry solvents were performed using oven-dried glassware. NMR experiments were performed on a Bruker DPX300 instrument. IR spectra were recorded on a Perkin-Elmer FT-IR 1600 spectrophotometer. Melting points (uncorrected) were determined on an Electrothermal IA9020 digital melting point apparatus. The microanalysis was performed by H. Kolbe Mikroanalytisches Laboratorium, Mühlheim an der Ruhr, Germany.

2-(2-Tosyloxyethyl)pyridine (10)

2-(2-Hydroxyethyl)pyridine (**11**) (1.23 g, 10 mmol) was dissolved in CH_2Cl_2 (20 mL). The solution was cooled to 0°C and triethylamine (2.02 g, 20 mmol) was added. *p*-Toluenesulfonyl chloride (2.86 g, 15 mmol) was added in portions during 10 min whereupon the cooling bath was removed. More and more precipitate was formed and after 24 h the reaction had gone to completion as judged by TLC (50 % ethyl acetate-petroleum ether). The thick mixture was diluted with CH_2Cl_2 (100 mL) and washed consecutively with water (30 mL) and brine (40 mL) before drying ($MgSO_4$). After filtering and evaporation of the organic solvents the residue was purified by column chromatography (ethyl acetate-petroleum ether gradient, 0-100 %) to yield 2.47 g (89 %) of a golden oil, which solidified in a refrigerator to a white solid (**10**), mp 41-42 °C (lit.,[13] 44°C). The substance should be stored in a freezer.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.45 (br d, 1H, J = 4.1 Hz), 7.71 (d, 2H, J = 8.3 Hz), 7.60 (dd, 1H, J = 7.7, 7.7 Hz), 7.30 (d, 2H, J = 8.2 Hz), 7.16 (d, 1H, J = 7.7 Hz), 7.14 (dd, 1H, J = 4.1, 7.7 Hz), 4.46 (t, 2H, J = 6.6 Hz), 3.14 (t, 2H, J = 6.6 Hz), 2.45 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.7 (s), 149.6 (d), 144.8 (s), 136.6 (d), 133.0 (s), 129.9 (d), 128.0 (d), 124.0 (d), 122.0 (d), 69.6 (t), 37.6 (t), 21.8 (q); IR (KBr) 1593, 1569, 1474, 1441, 1350, 1188, 1173, 1095, 978, 962, 912, 817, 778, 658, 565, 550 cm^{-1}.

2-Chloro-1-[2-(2-pyridinyl)ethyl]-1*H*-benzimidazole (8)

Method 1: Potassium *tert*-butoxide (0.249 g, 2.22 mmol) was suspended in dry DMF (5 mL) under N_2 and cooled to 0°C whereupon 2-chlorobenzimidazole (**9**)[12] (0.308 g, 2.02 mmol) dissolved in dry DMF (5 mL) was added dropwise during 5 min. The mixture was stirred for 2 h before pyridine tosylate (**10**) (0.510 g, 1.84 mmol) dissolved in dry DMF (5 mL) was added dropwise during 5 min. The ice-bath was removed and the mixture was stirred at room temperature for 48 h. The mixture was poured onto ice (30 g) and extracted with diethyl ether (4x40 mL). The collected organic phases were then washed consecutively with water (30 mL) and brine (50 mL) before being dried ($MgSO_4$). After filtering the organic solvent was evaporated and the residue purified by column chromatography (ethyl acetate-petroleum ether gradient, 0-100 %) to give 254 mg (54 %) of **8** as a viscous, greenish oil, which was characterized immediately.

Method 2: 2-(2-Hydroxyethyl)pyridine (**11**) (134 mg, 1.09 mmol) was dissolved in dry THF (10 mL) under N_2. Triphenylphosphine (357 mg, 1.36 mmol) and 2-chlorobenzimidazole (**9**)[12] (208 mg, 1.36

mmol) were added and the resulting suspension was cooled in an ice-bath to 0°C. DEAD (diethyl azodicarboxylate) (237 mg, 1.36 mmol) was added dropwise during 5 min giving a clear solution. After 2 h at 0°C consumption of starting alcohol was complete as judged by TLC (50 % ethyl acetate-petroleum ether) and the solution was diluted with ethyl acetate (50 mL). The organic phase was then extracted with 2 M aqueous hydrochloric acid (3×20 mL) to transfer the product into the aqueous phase. The collected aqueous phases were washed with two portions of ethyl acetate (15 mL) (to remove any remaining triphenylphosphine oxide) before basification (pH=9-10) with a 2 M aqueous sodium hydroxide solution. The product was now transferred to an organic phase by extracting with ethyl acetate (3×50 mL). The collected organic phases were washed with brine (60 mL) and then dried ($MgSO_4$). After filtering and evaporation of the solvents the residue was purified by flash chromatography with an ethyl acetate-CH_2Cl_2 gradient (0-100%) to give 131 mg (47 %) of a viscous, greenish oil, which was immediately characterized as **8**.
The compound (**8**) was characterized immediately after isolation due to its instability. When dissolved in $CDCl_3$ (for NMR) the solution quickly turned yellow and then green before a dark precipitate fell out. A cyclization to **6** (X=Cl) apparently began to take place, most probably catalyzed by traces of acidic impurities. However, **8** seemed to be infinitely stable in DMSO, so this appeared to be the solvent of choice for NMR studies. In neat form 7 underwent the same type of changes as in $CDCl_3$, even in a freezer.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.48 (d, 1H, J = 4.8 Hz), 7.63 (dd, 1H, J = 7.7, 7.7 Hz), 7.57 (d, 1H, J = 6.4 Hz), 7.49 (d, 1H, J = 6.6 Hz), 7.30-7.16 (m, 3H), 7.14 (d, 1H, J = 7.7 Hz), 4.62 (t, 2H, J = 7.0 Hz), 3.21 (t, 2H, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 157.3 (s), 149.1 (d), 141.0 (s), 139.7 (s), 136.5 (d), 134.8 (s), 123.5 (d), 122.8 (d), 122.2 (d), 121.9 (d), 118.5 (d), 110.4 (d), 43.8 (t), 36.8 (t). IR (neat) 1592, 1570, 1470, 1450, 1438, 1377, 743 cm^{-1}.

5,6-Dihydro-6a,11,11b-triazabenzo[*a*]fluorene hexafluorophosphate salt (6)

Method 1: **8** (254 mg, 0.99 mmol) was dissolved in MeCN (10 mL) and stirred at rt. The color of the solution changed to yellow, then to green with the concomitant formation of a dark precipitate. The mixture was stirred until the starting material was consumed (48 h) as judged by TLC (50 % ethyl acetate-petroleum ether). The solvent was then evaporated to give an almost pure chloride salt. The residue was now dissolved in water (40 mL) and small amounts of dark impurities were filtered off and discarded. The red-brown filtrate was washed with 3 portions of CH_2Cl_2 (10 mL) to remove any remaining starting material. $NaPF_6$ (250 mg, 1.49 mmol) was thereafter added to the aqueous phase and within seconds the greenish-looking **6** (X=PF_6) fell out. The precipitate was collected and dried in a desiccator to give 253 mg (70 %) of analytically pure, light yellow **6** (X=PF_6), mp 220-223°C.

Method 2: 2-(2-Hydroxyethyl)pyridine (**11**) (123 mg, 1.0 mmol) was dissolved in dry THF (10 mL) under N_2. Triphenylphosphine (328 mg, 1.25 mmol) and 2-chlorobenzimidazole (**9**)[12] (183 mg, 1.25 mmol) were added and to the resulting suspension was added dropwise DEAD (218 mg, 1.25 mmol) during 5 min, giving a clear solution. Gradually, the solution darkened as cyclization took place. After stirring for 48 h at rt the reaction mixture was transferred to a separation funnel with the help of CH_2Cl_2 (50 mL) and water (50 mL). The organic phase was discarded and the aqueous phase was washed with CH_2Cl_2 (3×10 mL). $NaPF_6$ (252 mg, 1.50 mmol) was added to the aqueous phase to precipitate the greenish-looking **6** (X=PF_6). The precipitate was collected and dried in an desiccator to give 240 mg (65 %) of analytically pure light yellow **6** (X=PF_6), mp 220-223°C.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.87 (br d, 1H, J = 6.4 Hz), 8.76 (dd, 1H, J = 7.9, 7.8 Hz), 8.31 (d, 1H, J = 7.9 Hz), 8.20 (dd, 1H, J = 7.1, 7.0 Hz), 7.86 (d, 1H, J = 8.3 Hz), 7.80 (d, 1H, J = 7.8 Hz), 7.51 (dd, 1H, J = 7.3, 7.8 Hz), 7.44 (dd, 1H, J = 8.2, 7.1 Hz), 4.62 (t, 2H, J = 6.8 Hz), 3.95 (t, 2H, J = 6.8 Hz). That the coupling constant of the doublet at 9.87 ppm does not correspond to any other may be a consequence of poor resolution: the broadness of the signal indicates unresolved $^4J_{HH}$ couplings. ^{13}C NMR (DMSO-d_6, 75 MHz) δ 151.7 (s), 148.5 (d), 141.9 (s), 139.8 (s), 138.9 (d), 133.9 (s), 128.4 (d), 126.3 (d), 125.0 (d), 124.2 (d), 120.0 (d), 111.5 (d), 37.1 (t), 27.2 (t). IR (KBr) 3099, 1620, 1576, 1536, 1487, 1433, 895, 836, 757, 558 cm^{-1}. Anal. Calcd for $C_{14}H_{12}N_3F_6P$: C 45.79, H 3.29, N 11.44. Found: C 45.56, H 3.38, N 11.26.

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