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A study of the structure–activity relationship of $GABA_A$–benzodiazepine receptor bivalent ligands by conformational analysis with low temperature NMR and X-ray analysis

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ABSTRACT

The stable conformations of $GABA_A$–benzodiazepine receptor bivalent ligands were determined by low temperature NMR spectroscopy and confirmed by single crystal X-ray analysis. The stable conformations in solution correlated well with those in the solid state. The linear conformation was important for these dimers to access the binding site and exhibit potent in vitro affinity and was illustrated for α5 subtype selective ligands. Bivalent ligands with an oxygen-containing linker folded back upon themselves both in solution and the solid state. Dimers which are folded do not bind to Bz receptors.

1. Introduction

The $GABA_A$/BzR complex contains a chloride ion channel which comprises part of the major inhibitory neurotransmitter system in the CNS.[1] This system regulates numerous neurological functions including convulsions, anxiety, and sleep activity, as well as memory and learning processes.[2–5] This membrane-bound heteropentameric protein polymer is composed principally of α, β, and γ subunits. Recombinant receptors containing these subunits closely mimic the biological, electrophysiological and pharmacological properties of native $GABA_A$ receptors.[5–7] Agents selective for specific BzR subtypes may permit one to separate out the pharmacological activities of these different isoforms.[8–12] This is a goal of paramount importance in the search for new anxiolytic agents and new anticonvulsant compounds with decreased side effects.[13–17]

Currently, transforming monomers into a bivalent ligand is one of the successful strategies for developing potent ligands with enhanced selectivity.[18–20] Bivalent ligands are defined as compounds which contain two pharmacophores joined through a connecting unit or linker. The general structure for bivalent ligands is described as P-X-P (P, pharmacophore; X, linker) (see Table 1). The proper selection of a suitable linker X is crucial for potent receptor binding.

Recent studies on the binding selectivity of the inverse agonist RY-80 (**1**) indicated preferential binding to α5 BzR/$GABA_A$ subtypes.[21,22] Therefore, the bivalent ligand Xli093 (**2**) was developed by incorporating the pharmacophore of **1** with a three-carbon linker.[22] This bivalent ligand exhibited selective affinity for the α5 subtype and behaved as a selective antagonist of the effects of diazepam in oocytes at this α5 subtype. Effects at the other three diazepam-sensitive sites were minimal. Encouraged by this, a

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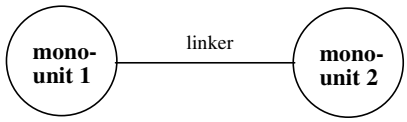
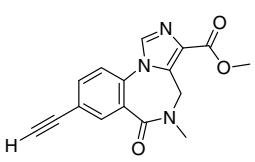
Table 1





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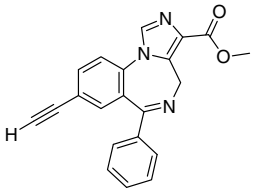

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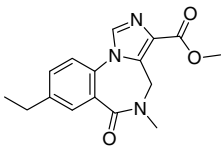


C

Compound	Monounit 1	Monounit 2	Spanner	Stable Conformation		K_i (αnβ3γ2) = nM					
				X-ray	NMR	α1	α2	α3	α4	α5	α6
1[a]	A	—	C_2H_5	—	—	28.4	21.4	25.8	5.3	0.49	2 8.8
2	A	A	$(CH_2)_3$	Linear	Linear	>1000	>1000	858	1550	15	>2000
3	A	A	$(CH_2)_5$	—	Linear	231	661	2666	ND	5.4	54.22
4	C	C	$(CH_2)_3$	—	Linear	1852	4703	8545	ND	100.5	5000
5	A	A	CH_2OCH_2	—	Folded	3795	2694	1864	ND	76.14	ND
6[a]	B	—	C_2H_5	—	—	287	45	96	1504	13.8	1000
7	B	B	$(CH_2)_2O(CH_2)_2$	Folded	Folded	460	5000	ND	ND	5000	5000
8	B	B	$(CH_2)_3$	Linear	Linear	236	7.4	272	>5000	194.2	>5000

[a] Monomer.

series of new bivalent ligands were designed and synthesized (Table 1).

It was hoped that these new dimers might exhibit enhanced selectivity and potency at the α5 BzR/$GABA_A$ subtypes. It was also expected that insertion of an oxygen atom into the linker might increase water solubility and hence enhance molecular hydrophilicity which should play an important role in the pharmacokinetic properties of the ligand.

The nature of the functional groups in a ligand plays an important role in receptor binding, of course, as well as the conformation in solution. The more information about the stable conformation(s) of molecules the better the understanding of the structure–activity relationships. It was essential from the beginning of the present study to determine the conformation of these bivalent ligands, which contained 3–5 atom linkers, since the steric requirements for affinity to the Bz receptor must be satisfied.[23]

In traditional medicinal chemistry, computer assisted molecular modeling programs and X-ray analysis contribute greatly in the search for stable conformations. However, some problems with these methods should not be neglected. Using computer modeling to determine the stable conformation of molecules containing many freely rotating bonds, such as those contained in bivalent ligands, is difficult. Although X-ray crystallography is the ultimate arbiter of chemical structure, it has many limitations beyond the obvious need for crystals: it often does not reflect accurately the conformation in solution, nor is it informative regarding conformational equilibria. This information is crucial in drug design. However, NMR spectroscopy is a powerful technique in drug discovery and its role in conformational analysis cannot be surpassed by other spectroscopic methods.[24]

Herein is described a method utilizing low temperature NMR for the determination of the solution stable conformation of a series of $GABA_A$–benzodiazepine bivalent ligands with different monomeric units and linkers. The conformations in solution were determined by NMR spectroscopy and compared with those in the crystal structure. The combination of low temperature NMR and X-ray analysis provided accurate structural information required for understanding structure–activity relationships and drug design. The influence of the molecular structure of the linker on the conformation is also discussed.

2. Results

Recently it was shown the active dimer **2** existed in a linear conformation in the solid state while dimer **7** with an oxygen-containing linker folded back upon itself, as illustrated in Figure 1.[25]

Since the bioactive conformation in solution may or may not parallel that in the crystal structure, the lowest energy solution structure must be established in order to correlate conformation with biological activity. Thus, NMR experiments at variable temperatures were performed and data were collected in different solvents. In methylene chloride or chloroform at room temperature, only a single set of signals was detected for both bivalent ligands **2** and **7**. At low temperature, it was found that the linear dimer **2** exhibited only a small splitting of about 3 Hz for some of the aromatic protons in the ^1H NMR spectra,[25] while two clearly separated sets of signals were observed for the folded dimer **7** (Fig. 2). For example, as seen in Figure 2 for **7**, the signal of H1 (7.92 ppm at 298 K) was split into two peaks at δ 7.91 and 7.88 ppm, respectively, at 193 K. Similar results were observed in the ^{13}C spectrum where C1 (134.9 ppm at 298 K) split into two signals at 135.3 and 135.4 ppm at 198 K. The doubling of the signals is consistent with disruption of the symmetry between the two domains of the molecule as expected if **7** adopted a static folded structure similar to the crystalline state.

However, the possibility could not be ruled out that the split in the signals was caused by slowing a dynamic process within each domain, such as conformational interconversion of the seven-membered ring. In order to investigate this possibility, the NMR spectra of the monomer **6** were run at low temperature as well. At temperatures as low as 173 K only one set of signals was observed in both the ^1H and ^{13}C NMR spectra of **6** (Fig. 3).[25] At lower temperatures, however, this was quite different from what was observed for dimer **7** at room temperature; the spectra of **6** and **7** were indistinguishable at 25 °C. Moreover, some addi-

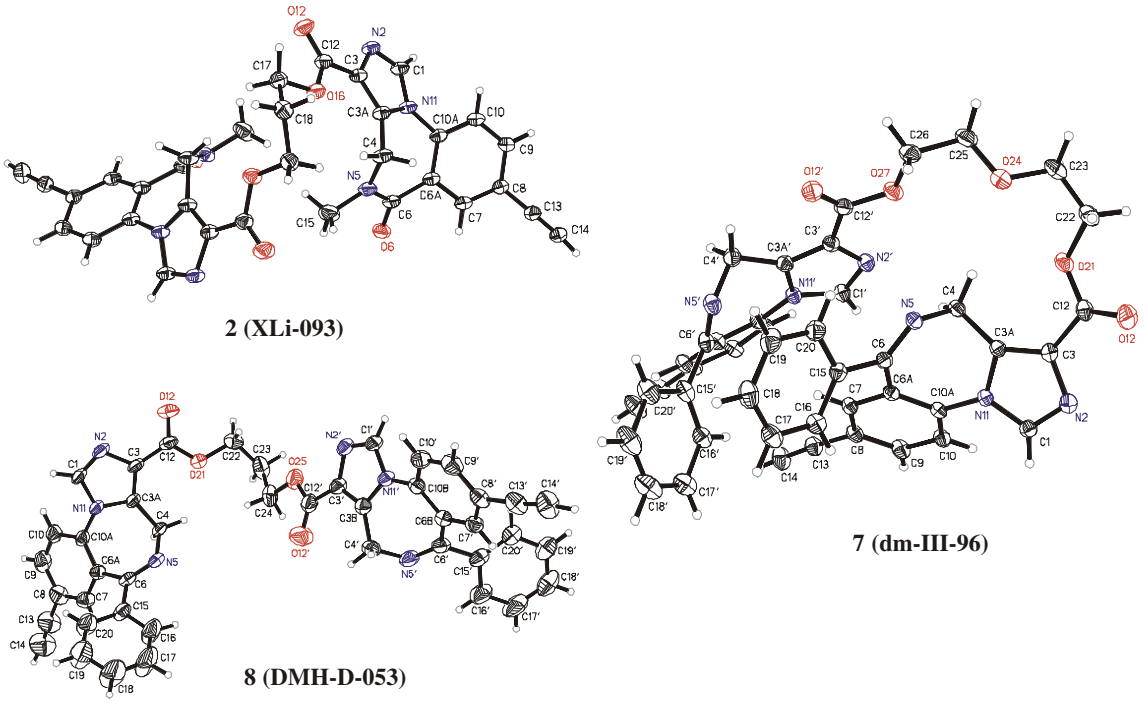


Figure 1. Crystal structure of 2 (left top), 7 (right) and 8 (left bottom).

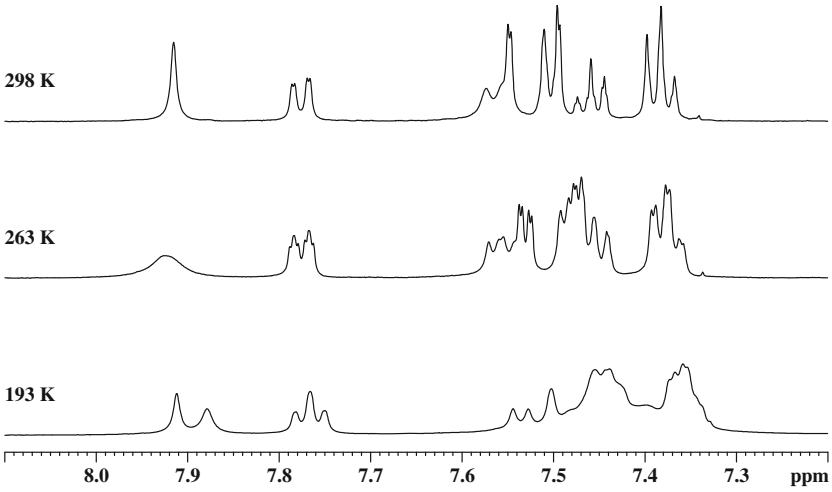


Figure 2. Aromatic region of ^{1}H NMR spectra of 7 in CD_2Cl_2 at variable temperatures.

tional line broadening of some of the aromatic signals was observed at the lowest temperature in both the monomer **6** and in its dimer **7**.

The analysis of these data indicated that the line broadening at the lowest temperature was due to one of the conformational processes mentioned above, whereas the doubling of the peaks in **7** was caused by the presence of two domains. Certainly an interdomain interaction existed between the two heterocyclic units of **7**, but not in monomer **6**. It was therefore concluded the internal mobility of the molecule decreased when the temperature was lowered which permitted observation of the two sets of signals of **7** on the NMR time scale. It was thus suggested that only when the molecule preferred the folded conformation in solution were two sets of signals observed. The preferred conformations of the molecules in $CDCl_3$ and CD_2Cl_2 correlated quite well with those observed in the crystal structures (Table 1).

The study was then expanded by varying the nature of the linker and monomer. Dimers **3** and **5** contain the same monomeric unit as **2**, whereas **8** contains an all carbon linker. It was found that **2**, **3, 4**, and **8** exhibited only one set of NMR signals at low temperature, whereas the NMR signals of ligands **5** and **7** split into two sets at low temperature. Low temperature NMR studies were performed in CD_2Cl_2. It was concluded that **5** and **7** preferred a folded conformation, while **2**, **3, 4**, and **8** assumed a linear conformation. These conclusions are supported by a crystal structure obtained for bivalent ligand **8** which indicated **8** was present in a linear conformation in the solid state. These results are illustrated in Table 1.

Since the goal was to design and synthesize bivalent ligands for biological applications in aqueous solution, the question arose: Do the conformations in $CDCl_3$ or CD_2Cl_2 resemble those in aqueous solution? Attempts to run the NMR experiments in water failed since the ligands were not sufficiently soluble in D_2O. However,

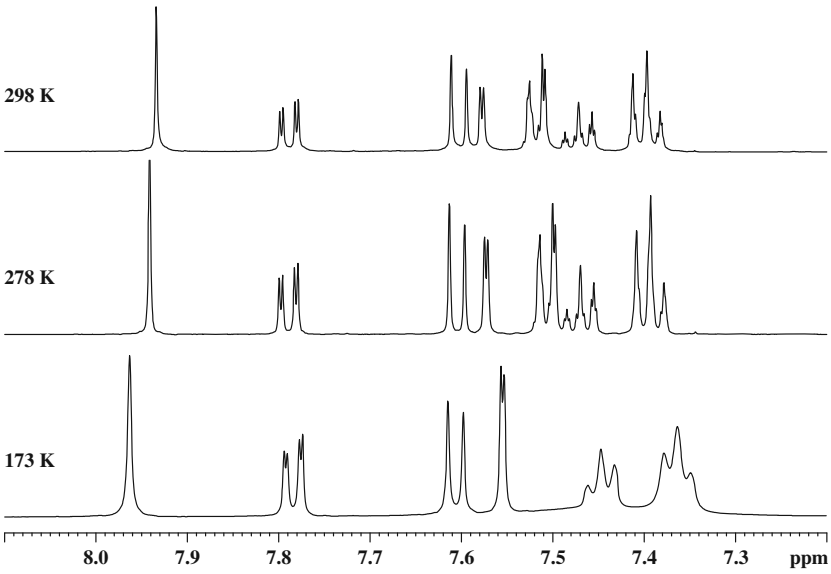


Figure 3. Aromatic region of ^{1}H NMR spectra of 6 in CD_2Cl_2 at different temperatures.

the spectra of both the linear and folded dimers **2** and **7** could be carried out in MeOH-d_4. The solvating properties of methanol, of course, more closely resemble those of water, and this more closely mimics aqueous physiological condition as well. The conformations of dimers in MeOH-d_4 were consistent with conformations in hydrophobic solvents.

3. Discussion

From the results described above, it is clear that dimers which contain an oxygen atom in the linker tend to adopt a folded conformation. Analysis of these data indicated in the hydrophilic solvent, **7** also had a higher tendency to fold back upon itself than **2**, as it did in the hydrophobic media (Figs. 4 and 5). In fact, the tendency of **7** to assume a folded structure appeared to be higher in methanol than in CD_2Cl_2 or $CDCl_3$, as the free rotation of the molecule was limited (Fig. 6). On the other hand, for dimer **2**, which preferred a linear structure in the solid state and in lipophilic solvents, only one set of signals was observed at room temperature in MeOH-d_4 (Fig. 7).

It is interesting to note that in the preparation of the samples, dimer **2** was less soluble in MeOH-d_4 than was **7**. It has been established that ligands are easier to dissolve in a solvent when the ligands surface energy can be minimized. Bivalent ligand **7** was more polar than dimer **2** and has more tendencies to fold back. Consequently, **7** was presumably, easier to dissolve in methanol because its surface energy was minimized. On the other hand, when ligand **2** was dissolved in a more polar solvent such as methanol, the surface energy may be forced to be minimized.

Since the conformation of molecules **2** and **7** in methanol agree with those in CD_2Cl_2 or $CDCl_3$, the behavior of these ligands in CD_2Cl_2 or $CDCl_3$ should reflect those in aqueous solution. The stable conformation of the compounds determined in CD_2Cl_2 or $CDCl_3$ were correlated with the newly generated receptor binding data

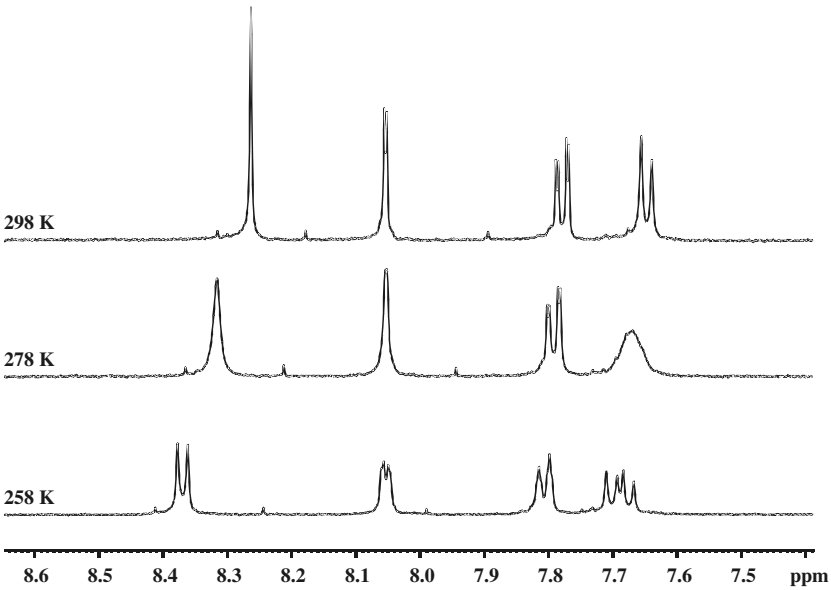


Figure 4. Aromatic region of ^{1}H NMR spectra of 2 in MeOH-d_4 at different temperatures.

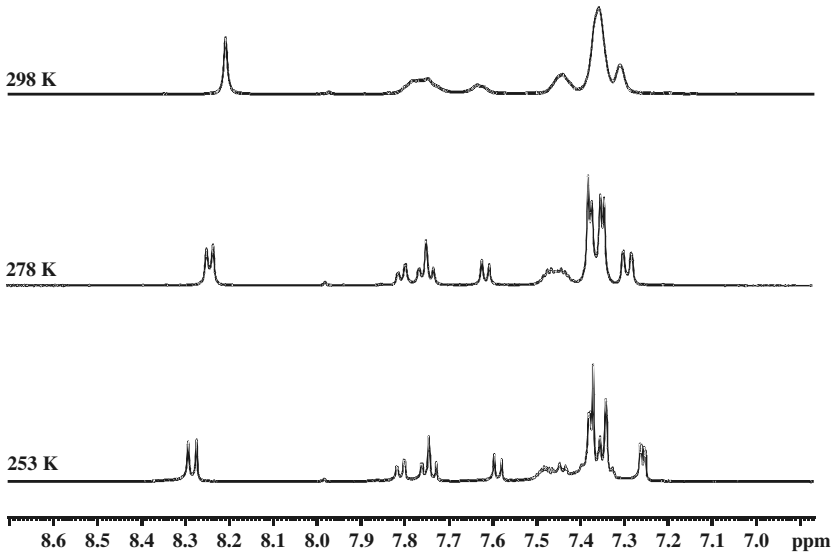


Figure 5. Aromatic region of proton spectra of 7 in MeOH-d_4 at different temperatures.

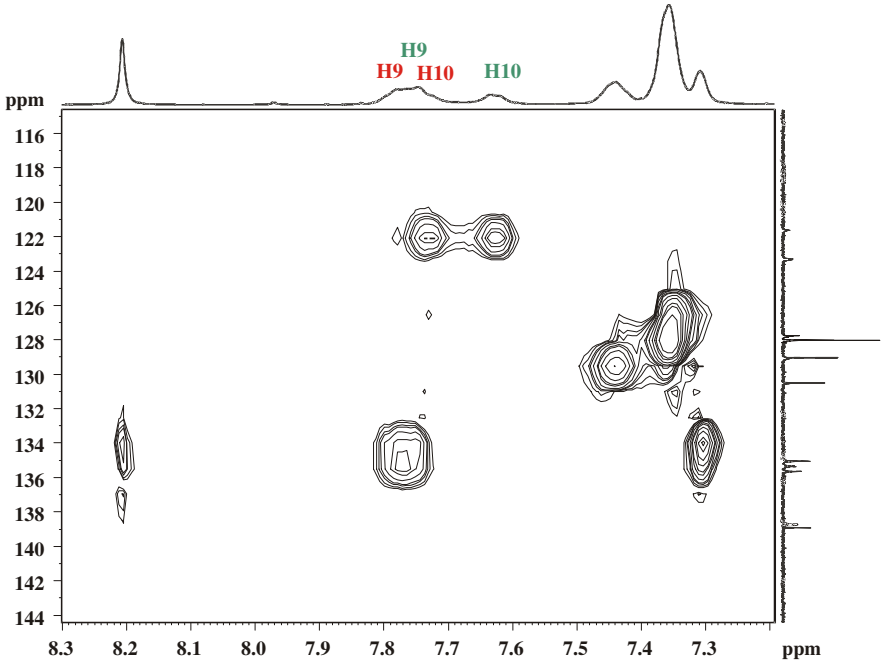


Figure 6. Partial HSQC spectrum of 7 in MeOH-d_4 at 298 K.

(Table 1). In the pharmacophore/receptor model, the bivalent ligands in the linear conformation align well (Fig. 7).

Importantly, bivalent ligands **2**, **3, 4**, and **8** with carbon only linkers preferred the linear conformation as the stable conformation, independent of the number of linker atoms. In contrast, replacing the middle carbon of either linker $(CH_2)_3$ or $(CH_2)_5$ with an oxygen atom altered the stable conformation of the molecules **5** and **7** from linear to folded. The only difference between bivalent pairs **2** and **5** with linear and folded conformations as the stable ones, respectively, was the center atom. In compound **2**, the middle atom was carbon, while in **5**, oxygen was present. Consequently, it was decided to focus attention on the conformational difference between carbon and oxygen-containing linkers.

It was well known[26,27] that the carbon chain in both small molecules and polymers favored the *anti* conformation which results in a linear arrangement of atoms. From examination of the linkers (Fig. 8), it was easily seen that linkers **C** and **D** can be regarded as oligomers of oxymethylene $(OCH_2)_2$ and oxyethylene $(OCH_2CH_2)_2$.

It was well documented that the preference for the *gauche–gauche* conformation[28–30] of a simple open-chain acetal such as dimethoxymethane ($CH_3OCH_2OCH_3$, **9**) could be predicted on the basis of the anomeric effect and related stereoelectronic effects. In this conformation the polar CO bonds are favorably oriented such that a lone pair orbital of the oxygen atom was almost antiperiplanar to the CO bond (Fig. 9, lone pair orbital and CO bond in same color). This permits maximum overlap of the n orbital of the oxygen atom with the σ^* orbital of the CO bond. This was not possible in the *anti–anti* or *gauche–trans* conformations and the two rabbit-ear interactions[28–32] (Fig. 9) engendered by each pair of adjacent oxygen atoms in the *anti-anti* conformer are avoided.

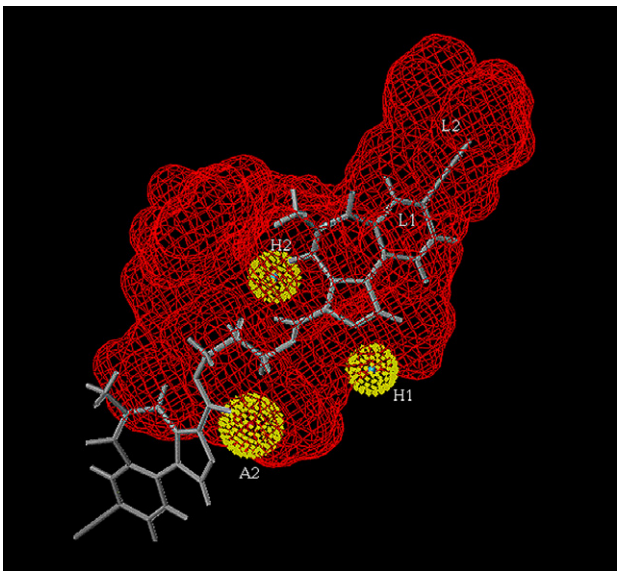


Figure 7. Bivalent ligand **2** (Xli093) aligned in the included volume of the pharmacophore/receptor model for the α5β3γ2 subtype.

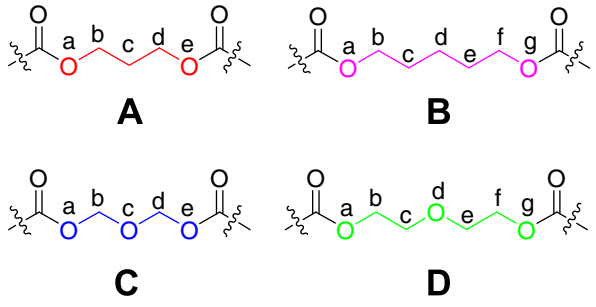


Figure 8. The linkers of the bivalent ligands.

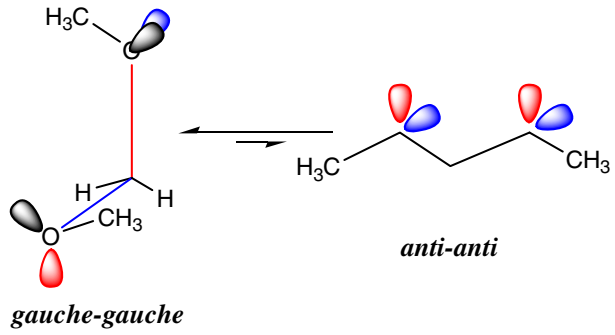


Figure 9. The conformations for dimethoxymethane.

Furthermore, in the related polymer of **9** with the two-bond repeating sequence, poly(oxymethylene) (**POM**), the *gauche* conformation was, in fact, markedly preferred over trans and the polymer existed in a helical (all *gauche*) conformation[27,33–35] rather than in the all-*anti* one.

Similarly, much effort has been spent on the investigation of the conformational characteristics of 1,2-dimethoxyethane (glyme, $CH_3OCH_2CH_2OCH_3$, **10**) as a model molecule for understanding the conformations of poly(oxyethylene) (**POE**). It had long been established that POE chains have a large fraction of bonds in *gauche* conformations and assumed a helical conformation overall.[26,27,33–35] It has been proposed that the oxygen gauche effect[27,36,37] and 1,5-CHO interaction[38–40] within the molecule were responsible for the *gauche*-rich conformations.

Based on this pioneering work, the correlation was made that the conformation of the linkers in the bivalents **25** and **78** adopt *anti* (**B**), *gauche–gauche* (**C**), *trans–gauche–trans* (**D**) conformations, respectively, regardless of the monomeric units which comprise them. The arrangement in space (disregarding the direction) of every unit in the linkers is depicted in Figure 10. The end-to-end distance of each unit (**C** and **D**) which adopted the *gauche* conformation was shorter than the one in the *anti* conformer. The more units in the linker, the shorter the *gauche* linker. Moreover, it was recognized that the CO bond length (1.43 Å) is often appreciably shorter than the CC bond (1.54 Å).[41] Therefore, it was believed that the linkers with the oxygen atom in the middle favored the helical conformation and rendered the two monomeric units in each dimer sufficiently close to each other with suitable dihedral angles to facilitate the intramolecular lipophilic–lipophilic (aromatic–aromatic) interaction. This was regarded as one of the most important factors to stabilize the folded structure as the preferred conformation.[42–46] For these same reasons, bivalent ligands with the linker B adopted the linear conformation.

For the higher analog of **POM** and **POE**, namely, poly(trimethylene oxide) [$(CH_2)_3O]_x$ (**POM₃**), *trans-gauche-gauche-trans* was sightly preferred over all-*trans* and *trans–trans–gauche–trans* conformers in the crystalline state.[27] The preference for the *gauche* state in this case was only 0.2 kcal/mol where as in the **POM** and **POE** examples was 1.5 and 0.4 kcal/mol, respectively.[33] Since the energy difference between *gauche* and *trans* was low, the linker **A** had more flexibility than the other linkers (**C** and **D**) to rotate freely and less tendency to occur as gauche. This could lead to improper end-to-end distances or dihedral angles for the interaction between the aromatic monounits. Hence, dimers connected with linker A could not be stabilized in the folded conformation even though the same aromatic monounits were contained in the molecules.

Bivalent ligands were evaluated in competition binding assays for specific $GABA_A$ membrane proteins using [^{3}H]flunitrazepam as the radiolabel. This assay measures the ability of the ligand to displace flunitrazepam. Data are reported as K_i according to the Cheng–Prusoff equation.[47] Biological data are presented in Table 1 for dimers synthesized using different spanners (or linkers). The linkers in **5** and **7** contained an oxygen atom. Compounds **2**, **3**, **4**, and **8** contained all carbon linkers. Binding data indicate decreased affinity when bivalents contain an oxygen atom in the linker. Compounds **2** and **3** showed increased selectivity for the α5 subtype as compared to parent monomer **1**. Compound **5** which was analogous to **2** with the exception of the oxygen atom present in the linker, bound with less affinity at the α5 subtype. Likewise bivalent **8** showed increased selectivity versus monomer **6**. However, the bivalent **7** containing an oxygen atom in the linker did not bind. The data suggest that dimers which contain a single oxygen atom in the linker bind with less decreased affinity to the Bz receptor. This is due to their propensity to adopt a folded conformation. A strategy to increase hydrophilicity and avoid a folded

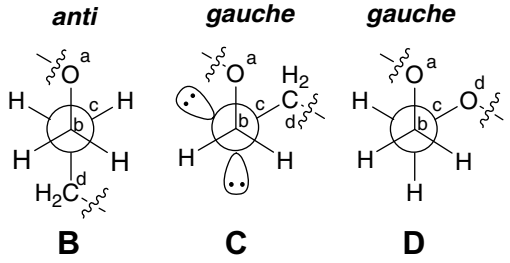


Figure 10. Newman projection for linkers B, C, and D.

conformation is to extend the linker length and insert two opposing oxygen atoms. This research is currently underway.

4. Conclusion

In summary, comparison of the results of low temperature NMR studies to crystal structures has provided enough information to demonstrate that low temperature NMR can be used as a quick method to identify dimeric ligands with a tendency to fold back upon themselves, as compared with those preferring a linear conformation. A correlation with binding data shows that the suitability of a ligand in the α5 BzR/Gabaergic subtype is heavily influenced by its conformation in solution. Variable temperature NMR thus can be used as a tool for screening bivalent ligands for their in vivo suitability. It is also clear the presence of one oxygen atom in the linker was the principle cause for the dimer to fold back onto itself. Ligands which contain two offsetting oxygen atoms in the linker are now under study in our laboratory.

5. Experimental

5.1. Synthesis

Inverse agonist **1** (RY080) was synthesized via the reported procedure.[3,48] Hydrolysis of the ester function of **1** provided the acid **9** in excellent yield and this material was subjected to a standard CDI-mediated coupling reaction to furnish bivalent ligands **2–5** in 60% yield (Scheme 1).[22,49]

The acid **11**, obtained from the ester **10**, which was available from the literature,[3,50] was stirred with CDI in DMF, followed by stirring with the required diol and DBU to provide bromide dimers **12** or **13**, respectively. They were converted into the trimethylsilylacetylenyl **14** or **15**, respectively, under standard conditions (Pd-mediated, Heck-type coupling).[51,52] The bisacetylene **7** or **8** (individually) was easily obtained by treatment of the trimethylsilyl ligand **14** or **15** with fluoride anion, as shown in Scheme 2.

5.2. Materials and general instrumentation

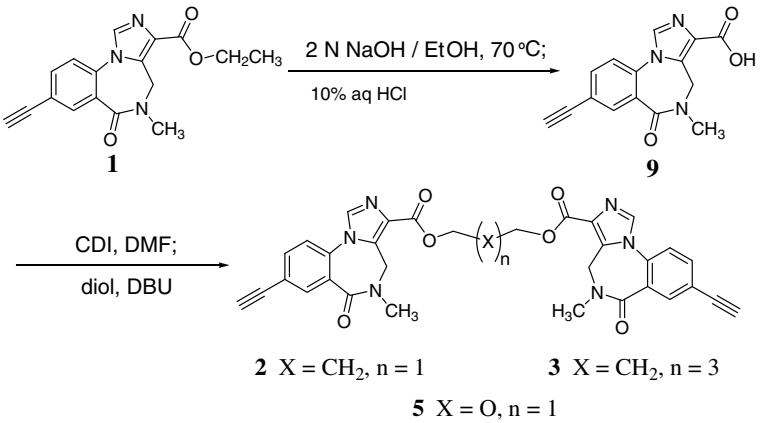
Chemicals were purchased from Aldrich Chemical Co. or Tokyo Chemical Industries and were used without further purification except where otherwise noted. Anhydrous THF was distilled from sodium/benzophenone ketyl. TLC analyses were carried out on Merck Kieselgel 60 F_{254}, and flash column chromatography was performed on silica gel 60b purchased from E.M. Laboratories. Melting points were taken on a Thomas–Hoover melting point apparatus or an Electrothermal Model IA8100 digital melting point apparatus and are reported uncorrected. NMR spectra were acquired on a Bruker DPX300 NMR spectrometer equipped with a z-gradient broadband (BBO) probe or on a Bruker DRX500 NMR spectrometer with either a triple axis gradient inverse (BBI) probe or a broadband observe (BBO) probe. Infrared spectra were recorded on a Nicolet DX FTIR BX V5.07 spectrometer or a Mattson Polaris IR-10400 instrument. Low-resolution mass spectral data (EI/CI) were obtained on a Hewlett–Packard 5985B GC–mass spectrometer, while high resolution mass spectral data were taken on a VG autospectrometer (Double Focusing High Resolution GC/Mass Spectrometer, UK). Microanalyses were performed on a CE Elantech EA1110 elemental analyzer.

5.3. Competition binding assays

Competition binding assays were performed in a total volume of 0.5 mL at 4 °C for 1 h using [^{3}H]flunitrazepam as the radioligand. For these binding assays, 20–50 mg of membrane protein harvested with hypotonic buffer (50 mM Tris–acetate, pH 7.4, at 4 °C) was incubated with the radiolabel as previously described.[53] Non-specific binding was defined as radioactivity bound in the presence of 100 μM diazepam and represented less than 20% of total binding. Membranes were harvested with a Brandel cell harvester followed by three ice-cold washes onto polyethyleneimine-pretreated (0.3%) Whatman GF/C filters. Filters were dried overnight and then soaked in Ecoscint A liquid scintillation cocktail (National Diagnostics; Atlanta, GA). Bound radioactivity was quantified by liquid scintillation counting. Membrane protein concentrations were determined using an assay kit from Bio-Rad (Hercules, CA) with bovine serum albumin as the standard.

5.4. Radioligand binding assays (Drs. McKernan and Atack)[49]

In brief, the affinity of compounds for human recombinant GABA(A) receptors was measured by competition binding using 0.5 nM [^{3}H]flunitrazepam. Transfected HEK Cells (β2 γ2 and desired α subtype) were harvested into phosphate-buffered saline, centrifuged at 3000g and stored at −70 °C until required. On the day of the assay, pellets were thawed and re-suspended in sufficient volume of 50 mM Tris/acetate (pH 7.4 at 4 °C) to give a total binding of approximately 1500–2000 dpm. Non-specific binding was defined in the presence of 100 mM (final concentration) diazepam. Test compounds were dissolved in DMSO at a concentration of 10 mM and diluted in assay buffer to give an appropriate concentration range in the assay, such that the final DMSO concentration in the assay was always less than 1%. Total assay volume was 0.5 mL and assays were carried out in 96-well plates and incubation time started by the addition of 0.1 mL of re-suspended cell membranes. Following incubation for 1 h at 4 °C, assays were terminated by filtration through GF/B filters, washed with 10 mL

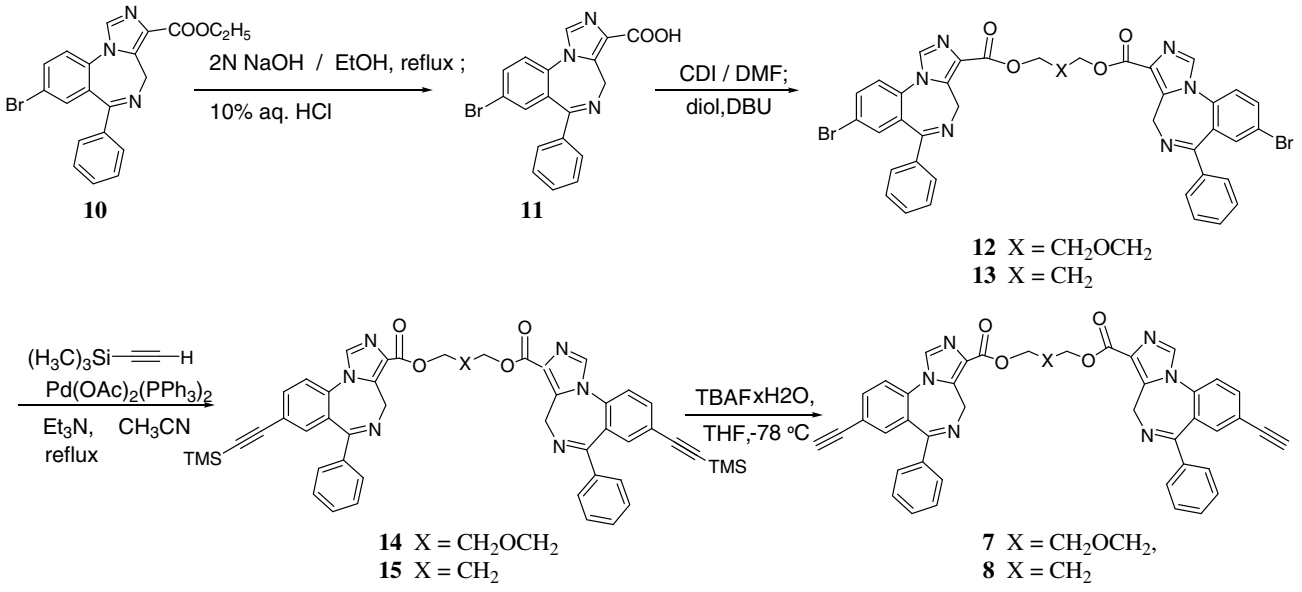


1 **9**

2 X = CH_2, n = 1 **3** X = CH_2, n = 3

5 X = O, n = 1

Scheme 1. Synthesis of bivalent analogs of Xli093.



Scheme 2. Synthesis of bivalent analogs of DMH-D-053.

ice-cold buffer, dried and then counted using a liquid scintillation counter. The percentage inhibition of [^{3}H]flunitrazepam binding, the IC_{50} and the K_i values were calculated using the Activity Base Software Package (ID Business Solutions, Guildford, UK) according to the Cheng–Prusoff equation.[47]

5.4.1. 1,3-Bis(8-acetyleno-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxy) propyl diester (2) (XLi093) (Procedure A)

To a solution of carbonyl diimidazole (230.3 mg, 0.57 mmol) in anhydrous DMF (5 mL) was added 8-ethynyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]-benzodiazepine-3-carboxylic acid **9** (200 mg, 0.71 mmol). The solution which resulted was stirred for 2 h at rt. Analysis by TLC (silica gel) indicated the absence of starting material. To the solution which resulted was then added 1,3-propanediol (27.1 mg, 0.36 mmol) in dry DMF (0.5 mL) and also DBU (114.2 mg, 0.75 mmol) in dry DMF (0.10 mL) at rt. The mixture was stirred at rt for 4.5 h until analysis by TLC (silica gel) indicated the reaction was complete. The reaction mixture was then poured into ice water (30 mL) and extracted with CH_2Cl_2 (3× 50 mL). The combined organic layer was washed with H_2O (5× 50 mL), brine and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/CH_3OH, 4:1) to provide **2** (157 mg) as a white solid in 73.4% yield. **2**: mp >230 °C (dec.); IR (NaCl) 3247, 1725, 1641, 1359, 1253, 1061 cm^{-1}; H NMR (300 MHz, $CDCl_3$) δ 2.37 (m, 2H), 3.24 (s, 2H), 3.26 (s, 6H), 4.40 (s, 2H), 4.57 (t, 4H, *J* = 6.2 Hz), 5.31 (br, 2H), 7.41 (d, 2H, *J* = 8.3 Hz) 7.72 (dd, 2H, *J* = 6.43, 1.86 Hz), 7.89 (s, 2H), 8.19 (d, 2H, *J* = 1.76 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.2, 34.4, 40.7, 60.2, 78.7, 79.7, 120.4, 121.6, 127.1, 127.7, 130.1, 133.4, 134.1, 134.9, 161.3, 164.1; MS (FAB, NBA) *m/e* (relative intensity) 603 (M^++1, 100). This material was employed for the X-ray crystal structure. It was homogenous in two independent TLC systems [R_f = 0.31 in EtOAc/CH_3OH, 4:1; R_f = 0.32 in CH_2Cl_2/CH_3OH, 9:1]. Anal. Calcd for $C_{33}H_{26}N_6O_6 \cdot 2/3\ CH_3OH$: C, 64.81; H, 4.63; N, 13.47. Found: C, 64.56; H, 4.72; N, 13.76.

5.4.2. 1,5-Bis(8-acetyleno-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxy) pentyl diester (3) (XLi210)

Ligand **3** was prepared by following the procedure A and acid **9** by replacing the 1,3-propanediol with 1,5-pentanediol to provide **3** as a white solid in 89.2% yield. **3**: mp 132–138 °C; IR (KBr) 3422, 3280, 2931, 1714, 1635, 1487, 1249, 1064 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.90 (m, 4H), 3.24 (s,6H), 3.52 (s, 2H), 4.39 (s, 8H), 5.29 (s, 2H), 7.36 (dd, 2H, *J* = 8.1, 16 Hz), 7.70 (m, 2H) 7.70 (m, 2H), 7.86 (s, 2H), 8.18 (s, 2H); MS (FAB, NBA) *m/e* (relative intensity) 631 (M^++1, 13). Anal. Calcd for $C_{35}H_{30}N_6O_6 \cdot 5/3\ H_2O$: C, 63.61; H, 4.83; N, 12.72. Found: C, 63.16; H, 4.72; N, 13.06.

5.4.3. 1,3-Bis(8-ethyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxy) propyl diester (4) (XLi356)

1,3-Bis(8-acetyleno-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]-benzodiaze-pine -3-carboxy) propyl diester **2** (500 mg, 0.83 mmol) was dissolved in EtOH (150 mL) after which Pd/C (176 mg) was added in solution at rt. The slurry was stirred for 5 h under one atmosphere of H_2 (bench top, balloon of H_2). The catalyst was removed by filtration and washed with EtOH. The EtOH was removed under reduced pressure to furnish a residue. This material was purified by flash chromatography (silica gel, EtOAc/EtOH 8:2) to provide **4** (504 mg, 99%) as white crystals: mp 125–133 °C; IR (NaCl) 3407, 2964, 2358, 1725, 1640, 1499 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (m, 6H), 2.39(m, 2H), 2.78 (dd, 4H, *J* = 7.5, 15.1 Hz), 3.26 (s, 6H), 4.48 (br, 2H), 4.56 (t, 4H, *J* = 6.1 Hz, 12.2 Hz), 5.16 (br, 2H), 7.33 (d, 2H, *J* = 8.2 Hz), 7.48 (d, 2H, *J* = 1.8 Hz), 7.89 (t, 4H, *J* = 3.2 Hz, 5.3 Hz), 8.15; MS(EI) *m/e* (relative intensity) 611 (M^++1, 100). Anal. Calcd for $C_{33}H_{34}N_6O_6 \cdot 2H_2O$: C, 61.33; H, 5.92; N, 13.00. Found: C, 61.74; H, 5.91; N, 12.63.

5.4.4. Bis(8-acetyleno-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxy) dimethyl glycol diester (5) (XLi374)

Ligand **9** (100 mg, 0.356 mmol) was dissolved in DMF (10 mL), and to this solution dibromodimethyl ether (36.3 mg, 0.178 mmol) was added, followed by TEA(2.0 mL). After 26 h of stirring at ambient temperature, the solvent was evaporated under reduced pressure and the residue was distributed between $CHCl_3$ and water. The organic phase was washed multiple times with water and dried over Na_2SO_4, and dried. The dried crude ether was applied on a column of silica gel to afford dimer 5 (86 mg) in 80% yield: mp >220 °C (dec.); IR (KBr) 3419, 3237, 2910, 1714, 1635, 1561,

1498 cm^{-1}; ^1H NMR (500 MHz, CD_2Cl) δ d 3.18 (s, 6H), 3.31 (s, 2H), 4.37 (br, 2H), 5.29 (br, 2H), 5.74 (s, 4H), 7.40 (d, 2H, J = 8.1 Hz), 7.74 (dd, 2H, J = 1.6, 8.2 Hz), 7.90 (s, 2H), 8.15 (s, 2H); MS(FAB,NBA) *m/e* (relative intensity) 605 (M^++1, 100). Anal. Calcd for $C_{32}H_{24}N_6O_7 \cdot 3/2\ CH_3COOC_2H_5$: C, 61.99; H, 4.93; N, 11.41. Found: C, 61.36; H, 4.52; N, 11.96.

5.4.5. 8-Bromo-6-phenyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylic acid (11)

The ester **10** (2 g) was dissolved in EtOH (50 mL) and aq sodium hydroxide (10 mL, 2 N) was added to the solution. The mixture was heated to reflux for 0.5 h. After the EtOH was removed under reduced pressure, the solution was allowed to cool. The pH value was adjusted to 4 by adding 10% aq HCl dropwise. The mixture was filtered and the solid was washed with water and ethyl ether. The solid was dried to provide **11** (1.8 g, 96.6%): mp >250 °C; IR (KBr) 3450 (b), 2844, 1707, 1615, 1493, 1166, 700 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 4.14 (d, 1H, J = 12.6 Hz), 5.79 (d, 1H, 12.6 Hz), 7.41–7.54 (m, 6H), 7.88 (d, 1H, J = 8.7 Hz), 8.03 (dd, 1H, J = 8.7, 2.1 Hz), 8.47 (s, 1H); MS (EI) *m/e* (relative intensity) 381 (M^+, 20), 383 (19).

5.4.6. 1,3-Bis(8-bromo-6-phenyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxy) propyl diester (13) (DMH-D-070) (Procedure B)

The carboxylic acid **11** (2 g, 5.2 mmol) was dissolved in DMF (20 mL), after which CDI (1.02 g, 6.3 mmol) was added at rt and the mixture was stirred for 2 h. Then 1,3-propanediol (0.19 mL, 2.6 mmol) and DBU (0.78 mL, 5.2 mmol) were added to the mixture and stirring continued overnight. The reaction solution was then cooled with an ice-water bath, after which water was added to precipitate a solid. This material was purified further by flash chromatography on silica gel (gradient elution, EtOAc/EtOH 20:1, 15:1, 10:1) to provide the bisbromide **13** (DMH-D-070) as a white solid (1.3 g, 61.9%): mp 187.5–189 °C; IR (KBr) 3112, 2968, 1708, 1610, 1559, 1491, 1269, 1160, 1123, 1073 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.35 (m, 2H), 4.08 (d, 2H, J = 12.6 Hz), 4.55 (m, 4H), 6.05 (d, 2H, J = 12.6 Hz), 7.37–7.53 (m, 12H), 7.6 (d, 2H, J = 2.1 Hz), 7.81 (dd, 2H, J = 2.1, 8.6 Hz), 7.93 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.2, 44.9, 61.4, 120.7, 124.2, 128.3, 129.0, 129.3, 129.6, 130.6, 134.1, 134.4, 134.7, 135.0, 138.9, 138.9, 162.6, 167.9; MS (FAB, NBA) *m/e* (relative intensity) 803 (M^++1, 15), Anal. Calcd for $C_{39}H_{28}N_6O_4Br_2$: C, 58.23; H, 3.51; N, 10.45. Found: C, 57.92; H, 3.43; N, 10.29.

5.4.7. 1,3-Bis(8-trimethylsilylacetylenyl-6-phenyl-4*H*-benzo-[*f*]imidazo[1,5-*a*][1,4]-diazepine-3-carboxy) propyl diester (15) (DMH-D-048) (Procedure C)

To a suspension of bisbromide **13** (1.005 g, 1.25 mmol) in acetonitrile (50 mL) and triethylamine (65 mL), was added bis(triphenylphosphine)-palladium (II) acetate (0.15 g, 0.2 mmol). The solution which resulted was degassed and trimethylsilylacetylene (0.7 mL, 5 mmol) was added after which it was degassed again (argon followed by vacuum). The mixture was heated to reflux and stirring maintained overnight. After removal of the solvent under reduced pressure, the residue was dissolved in CH_2Cl_2 and washed with water. 3-Mercaptopropyl functionalized silica gel (0.6 g) was added into the organic layer and stirring continued for 1 h. The silica gel/Pd complex was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (gradient elution, EtOAc/EtOH 20:1, 15:1, 10:1) to furnish the bistrimethylsilyl dimer **15** (DMH-D-048, 680 mg, 60.8%) as a white solid: mp 169–172 °C; IR (KBr) 3449, 2950, 1725, 1720, 1715, 1496, 1250, 1160, 1080, 847 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.25 (s, 18H), 2.35 (m, 2H), 4.05 (d, 2H, J = 12.6 Hz), 4.55 (m, 4H), 6.02 (d, 2H, J = 12.6 Hz), 7.37–7.55 (m, 14H), 7.75 (dd, 2H, J = 1.8, 8.4 Hz), 7.94 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ *0.3,* 28.3, 44.9, 61.4, 97.4, 102.3, 122.4, 122.6, 128.0, 128.3, 129.0, 129.4, 130.5, 134.1, 134.9, 135.1, 139.0, 139.2, 139.2, 162.6, 168.5; MS (FAB, NBA) *m/e* (relative intensity) 839 (M^++1, 100). Anal. Calcd for $C_{49}H_{46}N_6O_4Si_2$: C, 70.14; H, 5.53; N, 10.02. Found: C, 69.97; H, 5.35; N, 9.77.

5.4.8. 1,3-Bis(8-acetylenyl-6-phenyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxy)propyl diester (8) (DMH-D-053) (Procedure D)

A solution of bistrimethylsilyl dimer **15** (330 mg, 0.4 mmol) in THF (70 mL) was stirred with tetrabutylammonium fluoride hydrate (250 mg, 0.96 mmol) at −78 °C for 5 min. After this, H_2O (35 mL) was added to the solution to quench the reaction and stirring continued at low temperature for one half hour. The solution was extracted with EtOAc (3× 100 mL), and the organic layer was washed with water. After removal of the solvent under reduced pressure, ethyl ether was added to the residue to precipitate a solid. The mixture was filtered and the solid was washed with CH_2Cl_2/Et_2O (ca. 1:15) to provide the bisacetylenyl dimer **8** (DMH-D-053, 220 mg, 80%) as a yellow solid which crystallizes in CH_2Cl_2: mp 172–175 °C; IR (KBr) 3450, 3280, 2950, 1720, 1715, 1495, 1250, 1120, 1050 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.35 (m, 2H), 3.18 (s, 2H), 4.08 (d, 2H, J = 12.3 Hz), 4.56 (m, 4H), 6.04 (d, 2H, J = 12.6 Hz), 7.36–7.59 (m, 14H), 7.78 (dd, 2H, J = 8.4, 1.7 Hz), 7.95 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.8, 45.4, 61.9, 80.2, 81.3, 121.4, 122.7, 128.1, 128.3, 129.0, 129.3, 130.5, 134.2, 135.2, 135.3, 135.6, 138.9, 139.2, 162.6, 168.5; MS (FAB, NBA) *m/e* (relative intensity) 695 (M^++1, 100). Anal. Calcd for $C_{43}H_{30}N_6O_4 \cdot \frac{1}{4}CH_2Cl_2$: C, 72.63; H, 4.30; N, 11.75. Found: C, 72.36; H, 4.27; N, 11.36.

5.4.9. Bis(8-bromo-6-phenyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]-diazepine-3-carboxy) diethylene glycol diester (12) (DM-III-93)

Ligand **12** was prepared from acid **11**, under the same conditions employed in procedure B, by replacing 1,3-propanediol with diethylene glycol to yield a yellow solid (93.7%) **12**: mp 165–168 °C; IR (KBr) 3060, 2956, 1725, 1610, 1558, 1491, 1267, 1161, 1123, 1074 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.93 (t, 4H, J = 4.8 Hz), 4.06 (d, 2H, J = 12.6 Hz), 4.54 (m, 4H), 6.05 (d, 2H, J = 12.6 Hz), 7.39–7.50 (m, 12H), 7.57 (d, 2H, J = 2.7 Hz), 7.80 (dd, 2H, J = 2.1, 8.4 Hz), 7.90 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 44.9, 63.6, 69.0, 120.7, 124.2, 128.3, 129.0, 129.3, 129.6, 130.6, 134.1, 134.4, 134.6, 135.0, 138.9, 139.0, 162.5, 167.9; MS (FAB, NBA) *m/e* (relative intensity) 833 (M^++1, 5). Anal. Calcd for $C_{40}H_{30}Br_2N_6O_5 \cdot 0.15CHCl_3$: C, 56.72; H, 3.57; N, 9.88. Found: C, 56.61; H, 3.55; N, 9.92.

5.4.10. Bis(8-trimethylsilylacetylenyl-6-phenyl-4*H*-benzo-[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxy) diethylene glycol diester (14) (DM-III-94)

Ligand **14** was prepared from dibromide **12,** under the same conditions employed in Procedure C by replacing 1,3-propanediol with diethylene glycol to produce a yellow solid (49.5%) **14**: mp 205–208 °C; IR (KBr) 3433, 2960, 1730, 1700, 1612, 1493, 1255, 1169, 1120, 1071, 847 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.25 (s, 18H), 3.93 (t, 4H, J = 5.4 Hz), 4.04 (d, 2H, J = 12.6 Hz), 4.55 (m, 4H), 6.04 (d, 2H, J = 12.6 Hz), 7.37–7.53 (m, 14H), 7.74 (dd, 2H, J = 1.2, 8.4 Hz), 7.91 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ *0.3,* 45.0, 63.6, 69.0, 97.5, 102.4, 122.5, 122.7, 128.1, 128.3, 129.0, 129.4, 130.5, 134.2, 135.0, 135.1, 135.2, 139.1, 139.3, 162.7, 168.6; MS (FAB, NBA) *m/e* (relative intensity) 869 (M^++1, 100). Anal. Calcd for $C_{50}H_{48}N_6O_5Si_2 \cdot \frac{1}{4}H_2O$: C, 68.81; H, 5.60; N, 9.62. Found: C, 68.88; H, 5.66; N, 9.51.

5.4.11. Bis(8-acetylenyl-6-phenyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxy) diethylene glycol diester (7) (dm-III-96)

Ligand **7** was prepared from diester **14**, under the same conditions employed in procedure B, by replacing 1,3-propanediol with diethylene glycol to provide a yellow solid (81.6%) **7**: mp 173–177 °C; IR (KBr) 3432, 3280, 1720, 1715, 1496, 1254, 1175, 1120, 1074 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.12 (s, 2H), 3.93 (t, 4H, J = 4.5 Hz), 4.06 (d, 2H, J = 12.6 Hz), 4.55 (m, 4H), 6.05 (d, 2H, J = 12.6 Hz), 7.38–7.56 (m, 14H), 7.75 (dd, 2H, J = 8.4, 1.8 Hz), 7.91 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 45.0, 63.6, 69.0, 79.8, 81.3, 121.3, 122.7, 128.1, 128.3, 129.0, 129.3, 130.5, 134.2, 135.2, 135.3, 135.6, 139.0, 139.1, 162.6, 168.4; MS (FAB, NBA) *m/e* (relative intensity) 725 (M^++1, 63). Anal. Calcd for $C_{44}H_{32}N_6O_5 \cdot \frac{1}{4}EtOAc \cdot 3/2\ H_2O$: C, 69.89; H, 4.82; N, 10.87. Found: C, 70.12; H, 4.45; N, 10.58.

6. X-ray crystallographic data

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication Nos. 687205(DMH-D-053), 222395(Xli093), and 222396(DM-III-96). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0) 1223 336033 or email: deposit@ccdc.cam.ac.uk).

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References and notes

1. Squires, R. F.; Braestrup, C. *Nature* **1977**, *266*, 732.
2. Ninan, P. T.; Insel, T. M.; Cohen, R. M.; Cook, J. M.; Skolnick, P.; Paul, S. M. *Science* **1982**, *218*, 1332.
3. Wong, G.; Skolnick, P. *Eur. J. Pharmacol.—Mol. Pharmacol. Sect.* **1992**, *225*, 63.
4. Mendelson, W. B.; Cain, M.; Cook, J. M.; Paul, S.; Skolnick, P. *Science* **1983**, *219*, 414.
5. Venault, P.; Chapouthier, G.; de Carvalho, L. P.; Simiand, J.; Morre, M.; Dodd, R. H.; Rossier, J. *Nature* **1986**, *321*, 864.
6. Sieghart, W. *Pharmacol. Rev.* **1995**, *47*, 181.
7. Nayeem, N.; Green, T. P.; Martin, I. L.; Barnard, E. A. *J. Neurochem.* **1994**, *62*, 815.
8. Griebel, G.; Perrault, G.; Letang, V.; Granger, P.; Avenet, P.; Schoemaker, H.; Sanger, D. J. *Psychopharmacology* **1999**, *146*, 205.
9. Benson, J. A.; Low, K.; Keist, R.; Mohler, H.; Rudolph, U. *FEBS Lett.* **1998**, *431*, 400.
10. Rudolph, U.; Crestani, F.; Benke, D.; Brunig, I.; Benson, J. A.; Fritschy, J. M.; Martin, J. R.; Bluethmann, H.; Möhler, H. *Nature* **1999**, *401*, 796.
11. McKernan, R. M.; Rosahl, T. W.; Reynolds, D. S.; Sur, C.; Wafford, K. A.; Atack, J. R.; Farrar, S.; Myers, J.; Cook, G.; Ferris, P.; Garrett, L.; Bristow, L.; Marshall, G.; Macaulay, A.; Brown, N.; Howell, O.; Moore, K. W.; Carling, R. W.; Street, L. J.; Castro, J. L.; Ragan, C. I.; Dawson, G. R.; Whiting, P. J. *Nat. Neurosci.* **2000**, *3*, 587.
12. Möhler, H.; Fritschy, J. M.; Rudolph, U. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 2.
13. Liu, R. Y.; Zhang, P. W.; McKernan, R. M.; Wafford, K.; Cook, J. M. *Med. Chem. Res.* **1995**, *5*, 700.
14. Liu, R. Y.; Hu, R. J.; Zhang, P. W.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1996**, *39*, 1928.
15. Yu, S.; Ma, C. R.; He, X. H.; McKernan, R.; Cook, J. M. *Med. Chem. Res.* **1999**, *9*, 71.
16. Bailey, D. J.; Tetzlaff, J. E.; Cook, J. M.; He, X. H.; Helmstetter, F. J. *Neurobiol. Learn. Mem.* **2002**, *78*, 1.
17. Platt, D. M.; Rowlett, J. K.; Spealman, R. D.; Cook, J.; Ma, C. R. *Psychopharmacology* **2002**, *164*, 151.
18. Erez, M.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1982**, *25*, 847.
19. Portoghese, P. S.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Ronsisvalle, G.; Lipkowski, A. W.; Takemori, A. E.; Rice, K. C.; Tam, S. W. *J. Med. Chem.* **1985**, *28*, 1140.
20. Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Yim, C. B.; Ronsisvalle, G.; Tam, S. W.; Takemori, A. E. *J. Med. Chem.* **1986**, *29*, 1855.
21. Roth, B. 2006, unpublished results.
22. Li, X. Y.; Cao, H.; Zhang, C. C.; Furtmueller, R.; Fuchs, K.; Huck, S.; Sieghart, W.; Deschamps, J.; Cook, J. M. *J. Med. Chem.* **2003**, *46*, 5567.
23. Huang, Q.; He, X. H.; Ma, C. R.; Liu, R. Y.; Yu, S.; Dayer, C. A.; Wenger, G. R.; McKernan, R.; Cook, J. M. *J. Med. Chem.* **2000**, *43*, 71.
24. *Burger's Medicinal Chemistry & Drug Discovery*, 6th ed.; John Wiley & Sons Inc., 2003..
25. Han, D. M.; Forsterling, F. H.; Li, X. Y.; Deschamps, J. R.; Cao, H.; Cook, J. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1465.
26. Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; Wiley Interscience: NY, 1994.
27. Flory, P. J. *Statistical Mechanics of Chain Molecules*; Interscience Publishers: NY, 1968.
28. Lemieux, R. U. *Molecular Rearrangement*; Interscience Publishers: NY, 1964.
29. Eliel, E. L. *J. Acc. Chem. Res* **1970**, *3*, 1.
30. Graczyk, P. P.; Mikolajczyk, M. *Topics in Stereochemistry*; John Wiley & Sons, 1994.
31. Hutchins, R. O.; Kopp, L. D.; Eliel, E. L. *J. Am. Chem. Soc.* **1968**, *90*, 7174.
32. Eliel, E. L.; Giza, C. A. *J. Org. Chem.* **1968**, *33*, 3754.
33. Uchida, T.; Kurita, Y.; Kubo, M. *J. Polym. Sci.* **1956**, *19*, 365.
34. Abe, A.; Mark, J. E. *J. Am. Chem. Soc.* **1976**, *98*, 6468.
35. Ohsaku, M. *Macromolecules* **1978**, *11*, 970.
36. Wolfe, S. *Acc. Chem. Res.* **1972**, *5*, 102.
37. Jaffe, R. J.; Smith, G. D.; Yoon, D. Y. *J. Phys. Chem.* **1993**, *97*, 12745.
38. Bultinck, P.; Van Alsenoy, C.; Goeminne, A. *J. Phys. Chem.* **2001**, *105*, 9203.
39. Bultinck, P.; Goeminne, A.; Van de Vondel, D. *J. Mol. Struct.* **1999**, *467*, 211.
40. Glendening, E. D.; Feller, D.; Thompson, M. A. *J. Am. Chem. Soc.* **1994**, *116*, 10657.
41. Glasstone, S.; Laidler, K. J.; Eyring, H. *The Theory of Rate Processes*; McGraw-Hill: NY, 1941.
42. Azumaya, I.; Uchida, I.; Kato, T.; Yokoyama, A.; Tanatani, A.; Takayanagi, H.; Yokozawa, T. *Angew. Chem. Int. Ed.* **2004**, *43*, 1360.
43. Rashkin, M. J.; Waters, M. L. *J. Am. Chem. Soc.* **2002**, *124*, 1860.
44. Tsuzuki, S.; Honda, K.; Uchimaru, T.; Mikami, M.; Tanabe, K. *J. Am. Chem. Soc.* **2002**, *124*, 104.
45. Hobza, P.; Selzle, H. L.; Schlag, E. W. *J. Am. Chem. Soc.* **1994**, *116*, 3500.
46. Burley, S. K.; Petsko, G. A. *Science* **1985**, *229*, 23.
47. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
48. Fryer, R. I.; Schmidt, R. A.; Sternbach, L. H. *J. Pharm. Sci.* **1964**, *53*, 264.
49. Li, X. *Synthesis of Selective Ligands for GABAA/Benzodiazepine Receptors*; University of Wisconsin-Milwaukee: Milwaukee, 2004.
50. Sternbach, L. H.; Fryer, R. I.; Metlesics, W.; Reeder, E.; Sach, G.; Saucy, G.; Stempel, A. *J. Org. Chem.* **1962**, *27*, 3788.
51. Austin, W. B.; Bilow, N.; Kelleghan, W. J.; Lau, K. S. Y. *J. Org. Chem.* **1981**, *46*, 2280.
52. Heck, R. F. *Palladium Reagents in Organic Synthesis*; Academic Press, 1985.
53. Choudhary, M. S.; Craigo, S.; Roth, B. L. *Mol. Pharmacol.* **1992**, *42*, 627.