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Synthesis of two bi-functional ligands for the QUEST three-hybrid system

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Abstract

The synthesis is described for two bi-functional ligands (**1**) and (**2**) that have been used as chemical inducers of dimerization for QUEST,[1] a three-hybrid system designed to detect novel enzyme activity in vivo.

Recently, there has been much interest in protein–protein and protein–ligand interactions. While a number of methods exist to study these types of interactions, methods related to the yeast two- and three-hybrid systems have proven especially attractive. Protein–protein interactions can be studied using the yeast two-hybrid system[2] while protein–ligand interactions can be studied using the yeast three-hybrid system.[3] In the yeast two-hybrid system[2] (Fig. 1A), protein–protein interactions are detected based upon the dimerization of transcriptional proteins that produce a detectable signal.

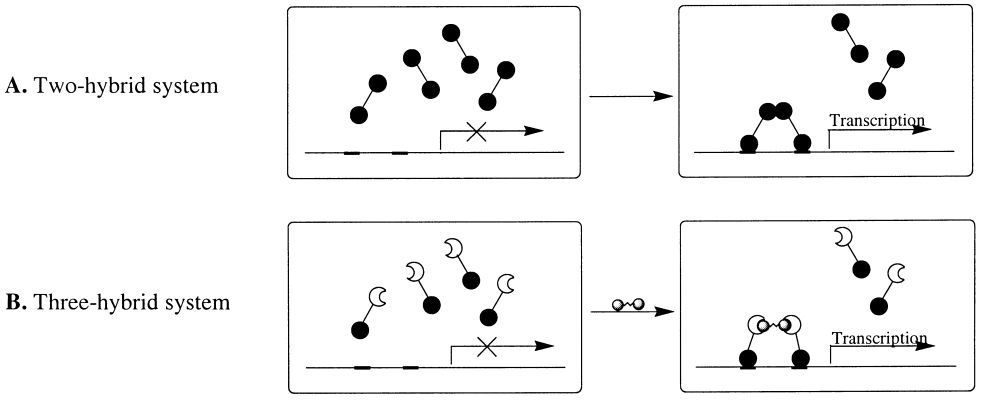

Figure 1.

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In the three-hybrid system[3] (Fig. 1B), the dimerization domain is replaced by a ligand-binding domain that is incapable of forming a dimer. Activation of the transcriptional event only occurs in the presence of a bi-functional ligand otherwise known as a chemical inducer of dimerization (CID), which results in the formation of an artificial dimer, causing transcription and an observable response. Recently, we published a derivative of the three-hybrid system, termed QUEST,[1] (QUerying for EnzymeS using the Three-hybrid system) which can detect novel enzymatic activities in vivo. Our initial target was to detect the fungal enzyme scytalone dehydratase in bacteria. To investigate this system, a mutant non-catalytic scytalone dehydratase (SD) was chosen as the ligand binding domain of the transcriptional protein. Two CIDs, (**1**) and (**2**) (Fig. 2), were designed for QUEST based upon two inhibitors of SD that have different K_i values. From an X-ray crystal structure[4] of SD, the ligands needed to be separated by at least 18Å in order to overcome steric requirements and effect dimerization of the DNA transcription proteins. To satisfy this parameter, the terminal ligands were separated by a central linking region that was 23Å in length. Here, we wish to report the synthesis of CIDs (**1**) and (**2**).

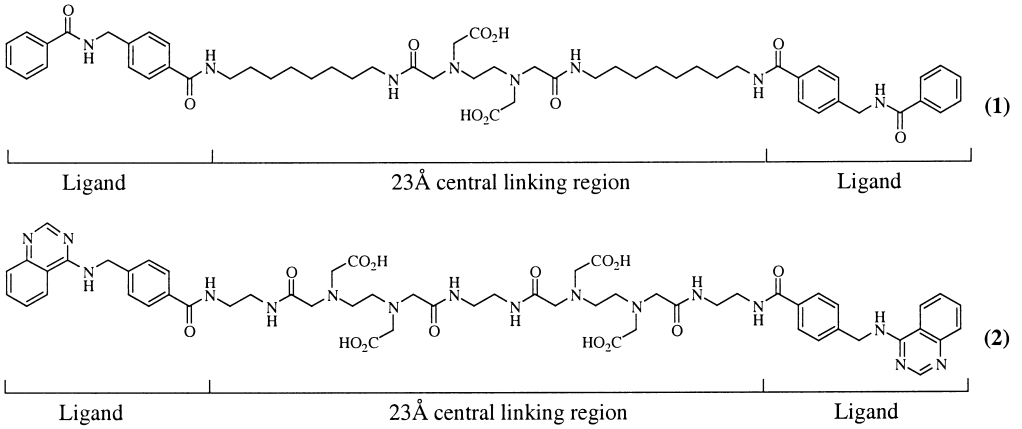
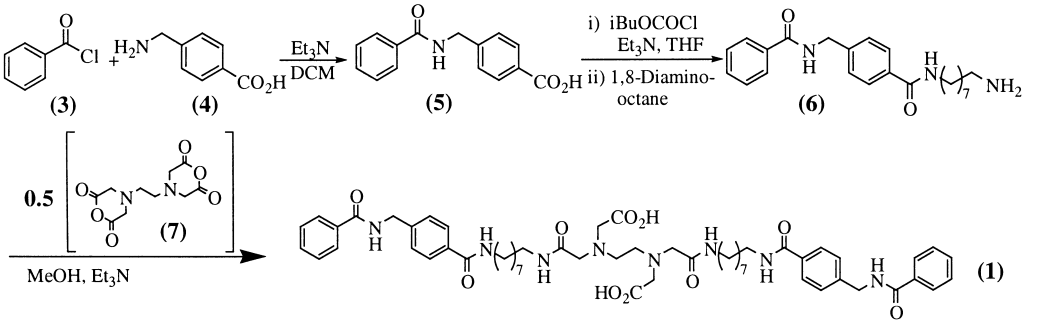


Figure 2.

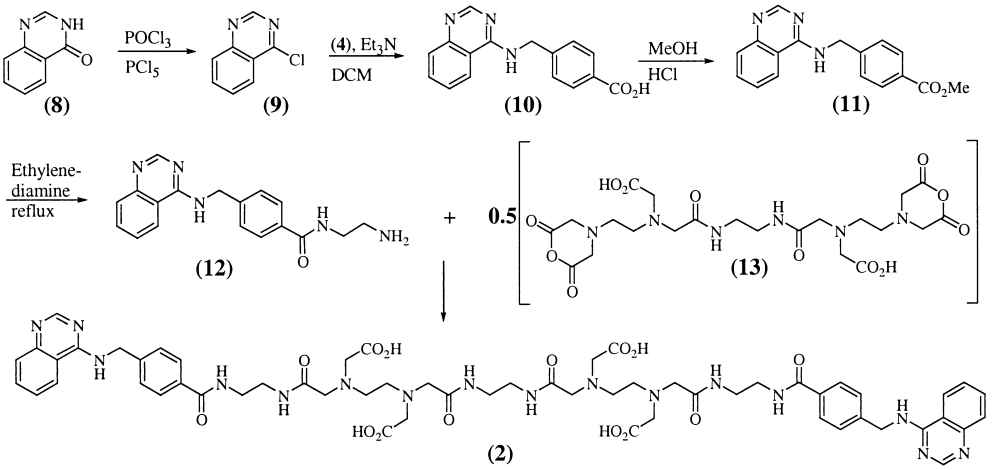
CID (**1**) was synthesized as described in Scheme 1. Benzoyl chloride (**3**) was treated with 4-aminomethylbenzoic acid (**4**) in dichloromethane with triethylamine to afford 4-benzoylamino-methylbenzoic acid (**5**)[5] in 46% yield.



Scheme 1.

The carboxylic acid group of compound (**5**) was then converted into a mixed anhydride using isobutylchloroformate in tetrahydrofuran with triethylamine, which was subsequently treated with 1,8-diaminooctane to furnish 4-benzoylaminomethyl-*N*-(aminooctyl)benzamide (**6**)[6] in 71% yield after column chromatography purification on silica gel. Two molar equivalents of compound (**6**) was then treated with 1 molar equivalent of ethylene diamine tetraacetic dianhydride (**7**) to complete the synthesis of ligand (**1**),[7] which was of sufficient purity to be used directly in the biological studies.

CID (**2**) was synthesized as described in Scheme 2. 4-Hydroxyquinazoline (**8**) was heated to reflux in phosphorus oxychloride with phosphorus pentachloride[8] to afford 4-chloroquinazoline (**9**) in 85% yield. This quinazoline (**9**) was subsequently treated with 4-aminomethylbenzoic acid (**4**) in *iso*-propanol with triethylamine to give 4-(*p*-carboxybenzyl)aminoquinazoline (**10**)[9] in 55% yield. The same methodology to synthesize the central linking region described for CID (**1**) was attempted but this was met with limited success. After conversion of the carboxylic acid of the 4-aminoquinazoline (**10**) to a mixed anhydride and subsequent treatment with 1,8-diaminooctane, we were unable to purify the product formed. This was due to the highly polar nature and low solubility of the product, which prevented us from separating it from the excess 1,8-diaminooctane used in the reaction. We decided to modify the central linking region to circumvent this problem. The carboxylic acid of the 4-aminoquinazoline (**10**) was converted to a methyl ester by using methanol saturated with hydrogen chloride giving 4-(*p*-methylbenzoate)aminoquinazoline (**11**)[10] in 92% yield. The methyl ester (**11**) was then heated to reflux in ethylene diamine to furnish 4-(*p*-*N*-(2-aminoethyl)benzamido)aminoquinazoline (**12**)[11] in quantitative yield. In the final step, 2 molar equivalents of ethylene diamine tetraacetic dianhydride was treated with 1 molar equivalent of diaminoethane in dimethylsulfoxide giving the dianhydride adduct (**13**). This was then treated with 2 molar equivalents of the aminoethylbenzamide (**12**) to complete the synthesis of ligand (**2**),[12] which was also of sufficient purity to be used directly in the biological studies.



Scheme 2.

Acknowledgements

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4. Lundqvist, T.; Rice, J.; Hodge, C. N.; Basarab, G. S.; Pierce, J.; Lindqvist, Y. *Structure* **1994**, *2*, 937–944.
5. Selected data for compound (**5**): ^{1}H NMR (CD_3OD, 360 MHz) δ 7.98 (d, $J=8.3$ Hz, 2H), 7.85 (m,2H), 7.55–7.43 (m, 5H), 4.63 (s, 2H); *m*/*z* (EI) 255 $[M]^+$; found: 256.0988, $C_{15}H_{13}NO_3$+H requires: 256.0904.
6. Selected data for compound (**6**): ^{1}H NMR (CD_3OD, 200MHz) δ 7.87–7.75 (m, 4H), 7.49–7.42 (m, 5H), 4.61 (s, 2H), 2.88 (t, $J=7.4$ Hz, 2H), 1.62 (br s, 4H), 1.38 (br s, 10H); *m*/*z* (FAB, Glycerol) 382 $[M+H]^+$; found: 382.2469, $C_{23}H_{31}N_3O_2$+H requires: 382.2495.
7. Selected data for compound (**1**): *m*/*z* (FAB, Glycerol) 1019 $[M+H]^+$, 1041 $[M+Na]^+$.
8. Endicott, M. M.; Wick, E.; Mercury, M. L.; Sherrill, M. L. *J. Am. Chem. Soc*. **1946**, *68*, 1299.
9. Selected data for compound (**10**): ^{1}H NMR (CD_3OD, 360 MHz) δ 8.63 (s, 1H), 8.29 (d, $J=8.7$ Hz, 1H), 7.92 (t, $J=7.8$ Hz, 1H), 7.85 (d, $J=8.3$ Hz, 2H), 7.71–7.65 (m, 2H), 7.39 (d, $J=8.2$ Hz, 2H), 4.96 (s, 2H); *m*/*z* (CI) 279 $[M]^+$; found: 280.1074, $C_{16}H_{13}N_3O_2$+H requires: 280.1086.
10. Selected data for compound (**11**): ^{1}H NMR (CD_3OD, 200 MHz) δ 8.79 (s, 1H), 8.44 (d, $J=8.7$ Hz, 1H), 8.11–7.98 (m, 3H), 7.86–7.79 (m, 2H), 7.55 (d, $J=8.2$ Hz, 2H), 5.11 (s, 2H), 3.87 (s, 3H); *m*/*z* (CI) 294 $[M+H]^+$; found: 294.1234, $C_{17}H_{15}N_3O_2$+H requires: 294.1243.
11. Selected data for compound (**12**): ^{1}H NMR (CD_3OD, 200 MHz) δ 8.41 (s, 1H), 8.18 (d, $J=8.6$ Hz, 1H), 7.83–7.68 (m, 4H), 7.53 (m, 1H), 7.47 (d, $J=8.4$ Hz, 2H), 4.90 (s, 2H), 3.46 (t, $J=6.2$ Hz, 2H), 2.84 (t, J = 6.5 Hz, 2H); *m*/*z* (FAB, 3-NBA) 322 $[M+H]^+$, 344 $[M+Na]^+$, 643 $[2M+H]^+$; found: 322.1657, $C_{18}H_{19}N_5O$+H requires: 322.1668.
12. Selected data for compound (**2**): *m*/*z* (FAB, 3-NBA) 1215 $[M+H]^+$.