HHS Public Access

Author manuscript

*Org Lett*. Author manuscript; available in PMC 2016 August 06.

Published in final edited form as:

*Org Lett*. 2009 September 17; 11(18): 4052–4055. doi:10.1021/ol901478x.

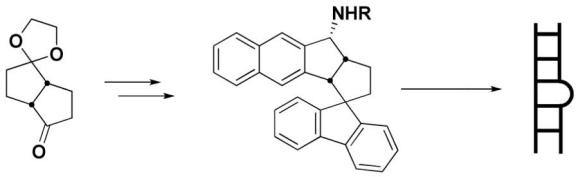
# Small Molecule Ligands for Bulged RNA Secondary Structures

**S. Todd Meyer** and **Paul J. Hergenrother***

Department of Chemistry, Roger Adams Laboratory, University of Illinois, Urbana, Illinois 61801

## Abstract

A class of wedge-shaped small molecules has been designed, synthesized, and shown to bind bulged RNA secondary structures. These minimally cationic ligands exhibit good affinity and selectivity for certain RNA bulges as demonstrated in a fluorescent intercalator displacement assay.




While the vast majority of drugs are ligands for proteins, the targeting of nucleic acids could emerge as a complementary approach. In particular, cellular RNA is an attractive and underexploited target for small molecule therapeutics.[1] The broad utility of RNA interference and the clinical success of ribosome-binding antibiotics hints at the potential uses for compounds that bind selectively to RNA.

The propensity of RNA to adopt hairpin loops, internal loops, and bulged secondary structures provides a unique opportunity for specific recognition by small molecule ligands.[1a] Thus, one method to target RNA is to identify individual small molecule "modules" that bind to specific RNA secondary structural elements, and combine them for selective RNA targeting. As a complement to compounds that bind selectively to RNA hairpin loops,[2] herein we disclose a novel class of ligands selective for bulged RNA secondary structures.

Our investigations in this area were inspired by the thiol-independent breakdown product of neocarzinostatin (**NCSi-gb**, Figure 1),[3] as well as by synthetic analogues developed by Goldberg and coworkers, such as **DDI** (Figure 1).[4] Derivatives of **NCSi-gb** and **DDI** possess a unique geometry that enables strong and reasonably selective binding to bulged regions of DNA, and one report shows that some **DDI** derivatives have moderate affinity for certain RNA bulges.[4e] We initially sought to derivatize the **DDI** spirocyclic alcohol in the preparation of a collection of RNA-binding compounds. However, certain **DDI** derivatives

* hergenro@uiuc.edu.

**Supporting Information Available:** Experimental procedures, binding data and spectral data for all new compounds, as well as X-ray structural data for **2** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

are susceptible to decomposition via a proposed retro-aldol pathway,[4f,5] and indeed we found that **1** (Figure 1) was highly sensitive to strong bases. Certain acylated derivatives of **1** were also unstable in aqueous environments. We thus sought a more hydrolytically stable scaffold that would be amenable to broad-scale derivatization.

The key feature of **NCSi-gb** and **DDI** that imbue them with their affinity for nucleic acid bulges was determined by Goldberg and co-workers to be the disposition of the aromatic rings, which form a wedge-like structure. This conformation is governed by the geometry of the benzobicyclo-[3.3.0]-octane core, which confers on **1** and its derivatives a twist angle of approximately 35°.[4a,c,d,f] We therefore designed ketone **2** (Figure 1), which was suggested by computational modeling to have a geometry similar to that of **1**. The limited oxygenation of **2**, as compared to **1**, was expected to lead to enhanced stability of the core scaffold.

The synthesis of **2** commenced from the known ketone **3** (Scheme 1),[6] which was used as the racemate. The reaction of **3** with 2-lithiobiphenyl, prepared from the corresponding iodide, afforded tertiary alcohol **4**. Treatment of **4** with concentrated hydrochloric acid in glacial acetic acid cleanly effected its dehydration with concomitant cleavage of the ketal, affording ketone **5**. Dehydrogenation of **5** to enone **6** was readily accomplished through a modification of the Nicolaou method.[7] The naphthyl ring system was introduced through a Diels-Alder cyclization-aromatization sequence,[8] which produced ketone **7**. Finally, Lewis acid-mediated cycloisomerization of **7** proceeded smoothly to afford the scaffold compound **2**. The structures of **2** and **7** were verified through single-crystal X-ray analysis. Gratifyingly, **2** was shown to adopt the desired wedge shape: the angle between the noncoplanar aromatic ring systems was determined to be 38.43° ± 0.12° (Figure 2). The overall synthetic sequence outlined in Scheme 1 is facile and permits rapid access to appreciable quantities of **2**, a critical factor that enabled the preparation of multiple lead compounds based on this scaffold.

Given that most RNA-ligand interactions are governed by electrostatic contacts between the small molecule and the phosphate backbone of the oligonucleotide,[1] it was hypothesized that these synthetic ligands would require some cationic functionality in order to facilitate binding. A two step reductive amination method was therefore developed to prepare a series of amine derivatives (**8**-**13**, Scheme 2). Ketone **2** was treated with various amines in the presence of excess $Ti(O\mathit{i}Pr)_4$ and 5Å molecular sieves to afford the corresponding imines, which were reduced through the action of sodium borohydride. This reductive amination approach enabled the introduction of structural diversity through reaction with dissimilar amines. In an effort to assess the importance of the spirocycle, several similarly substituted derivatives of **7** were also prepared under these conditions. These compounds (**14**-**16**, Scheme 3) retain the overall wedge shape exhibited by **2**, as evidenced by the crystal structure of **7** in Figure 2, but lack the spirocyclic junction.

Amines derived from **2** and **7** were isolated as single, racemic diastereomers (shown in Schemes 2 and 3), with stereochemical assignments made based on coupling constant analysis and NOESY studies. These compounds were found to be soluble to at least 50 μM in buffer with 5% DMSO, and stable at room temperature in assay buffer for greater than 24 hours, as determined by HPLC.

We also sought to investigate the importance of the wedge shape in the RNA-ligand binding interaction. As this aspect of the molecular architecture is controlled by the geometric constraints of the bicyclo-[3.3.0]-octane core, ketone **17**[8] was used to prepare an additional subset of amines (**18**-**20**, Scheme 4) lacking this structural motif. A compound containing a guanidinium moiety was also prepared to assess the influence of cationic character on binding relative to molecular geometry (Scheme 5). The reductive amination procedure was carried out on ketone **17** using monoprotected diamine **21**[9] to give **22**. Following Boc deprotection, the free amine was treated with Goodman's guanidinylating reagent **23**[10] in the presence of triethylamine to afford **24**, with subsequent deprotection revealing the guanidinium compound **25**.

The synthetic compounds were then assessed against a panel of RNA constructs containing one-, two-, or three-base bulges (**I**-**III**, Figure 3) to determine their binding properties. Duplex RNA **IV** was used as a control to identify ligands that were nonspecific RNA binding compounds. Ligand binding to RNA was determined using a fluorescent intercalator displacement assay.[11] Each RNA construct was incubated with ethidium bromide to form a fluorescent complex. Subsequent treatment with ligand led either to a reduction in the fluorescence of the complex, indicating binding of the ligand to the RNA construct, or no response. Compounds that displaced ethidium bromide from an RNA construct were subsequently evaluated for dose-dependent fluorescence quenching. These data were fit to a single-site binding model by curve-fitting analysis to determine the dissociation constant ($K_d$) of the interaction.

Several of the amine compounds bind to RNA bulges with good affinity and selectivity, while none of the ketones (**2**, **7**, **17**) display any binding affinity (Table 1). Among the spirocyclic series, **11**, **12** and **13** exhibit low-micromolar affinity for certain bulge sizes, with **13** having an apparent preference for the single-base bulge of construct **I**. Interestingly, the nonspirocyclic isomers of **11** and **12** (**14** and **15**, respectively) exhibit similar bulge-binding activities, suggesting that the spirocyclic junction is not required to lock the compounds in the appropriate binding conformation, but that the compounds may nevertheless adopt a similar conformation in the presence of RNA. Indeed, the X-ray crystal structures of **2** and **7** are strikingly similar. However, the lack of any binding by compounds **18**-**20** implicates the wedge shape, or at least the additional aromatic unit, as important to governing the binding conformation. Interestingly, not even the guanidinium group of **25** is sufficient to permit binding to the RNA constructs. Thus, while the nature of the substituent on the amine does appear to exert some influence on the ability of these compounds to bind RNA bulges, the overall shape of the molecule is a key factor enabling binding.

The compounds described herein constitute a new class of easily synthesized RNA-binding agents that are stable in aqueous buffer. Perhaps most importantly, these compounds are minimally cationic yet exhibit clear RNA-binding activity. Through evaluation of these compounds, we have demonstrated that the spirocycle of **2** is not required for RNA bulge binding *per se*, as non-spirocyclic versions that retain the twist geometry (**14** and **15**) are still RNA ligands.

Most small molecule ligands for RNA are polycationic (often containing amino sugars), with increasing cationic character typically leading to attenuated target selectivity.[1] The minimally charged nature of the compounds described in this work demonstrate the potential advantage of shape-based binding to RNA secondary structures. With a facile synthetic route to **2** now in place, multiple derivatives can be created in an effort to probe the sequence-specificity of these compounds, and to find compounds with even stronger affinities and selectivities for one-, two-, and three-nucleotide RNA bulges.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgment

The authors thank Jason R. Thomas (UIUC) for helpful conversations and Dr. Scott R. Wilson (UIUC) for assistance with X-ray crystallography. This work was supported by the University of Illinois and the National Institutes of Health (R01AR058361).

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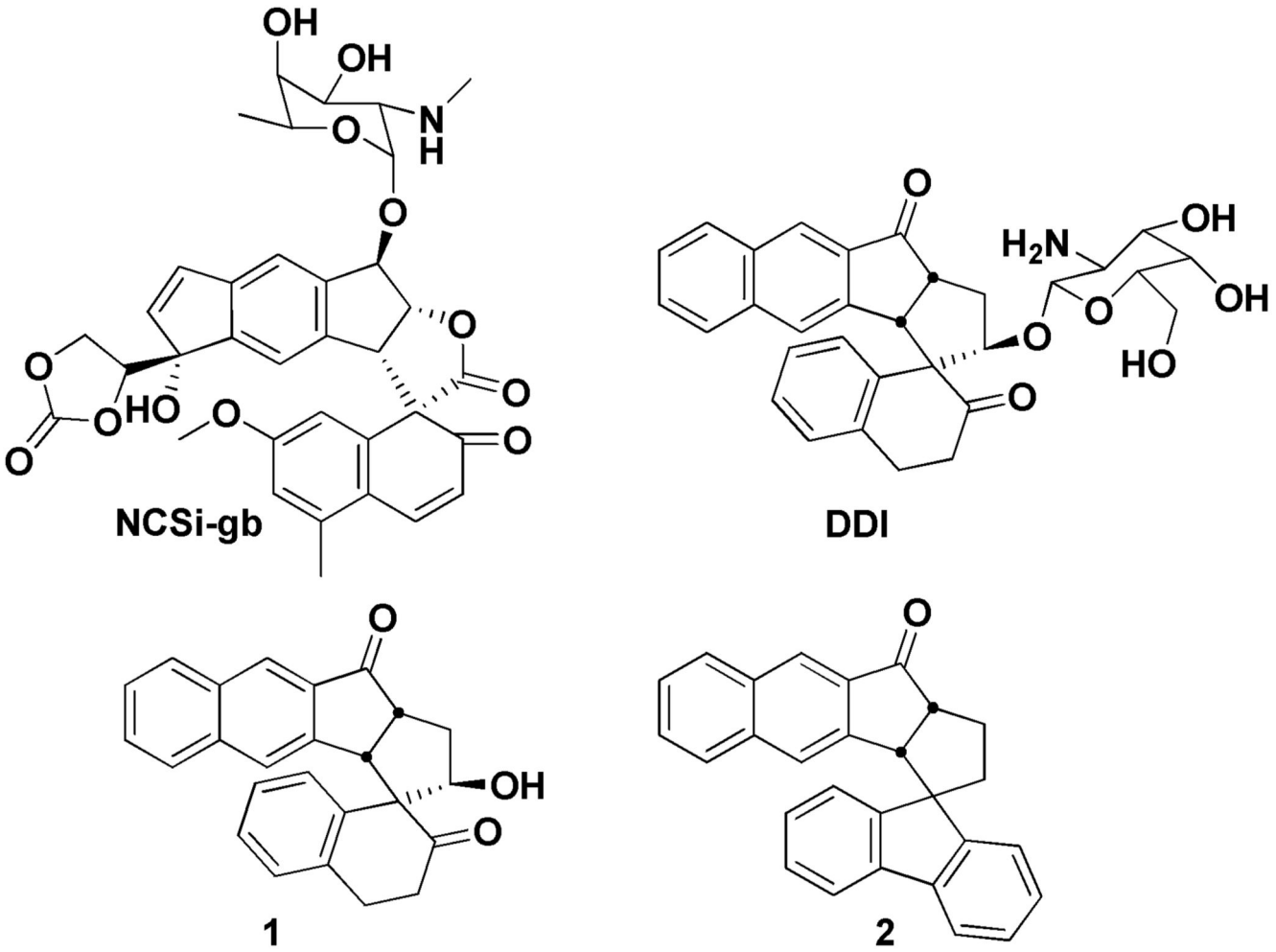



**Figure 1.**
Spirocyclic compounds for RNA binding studies

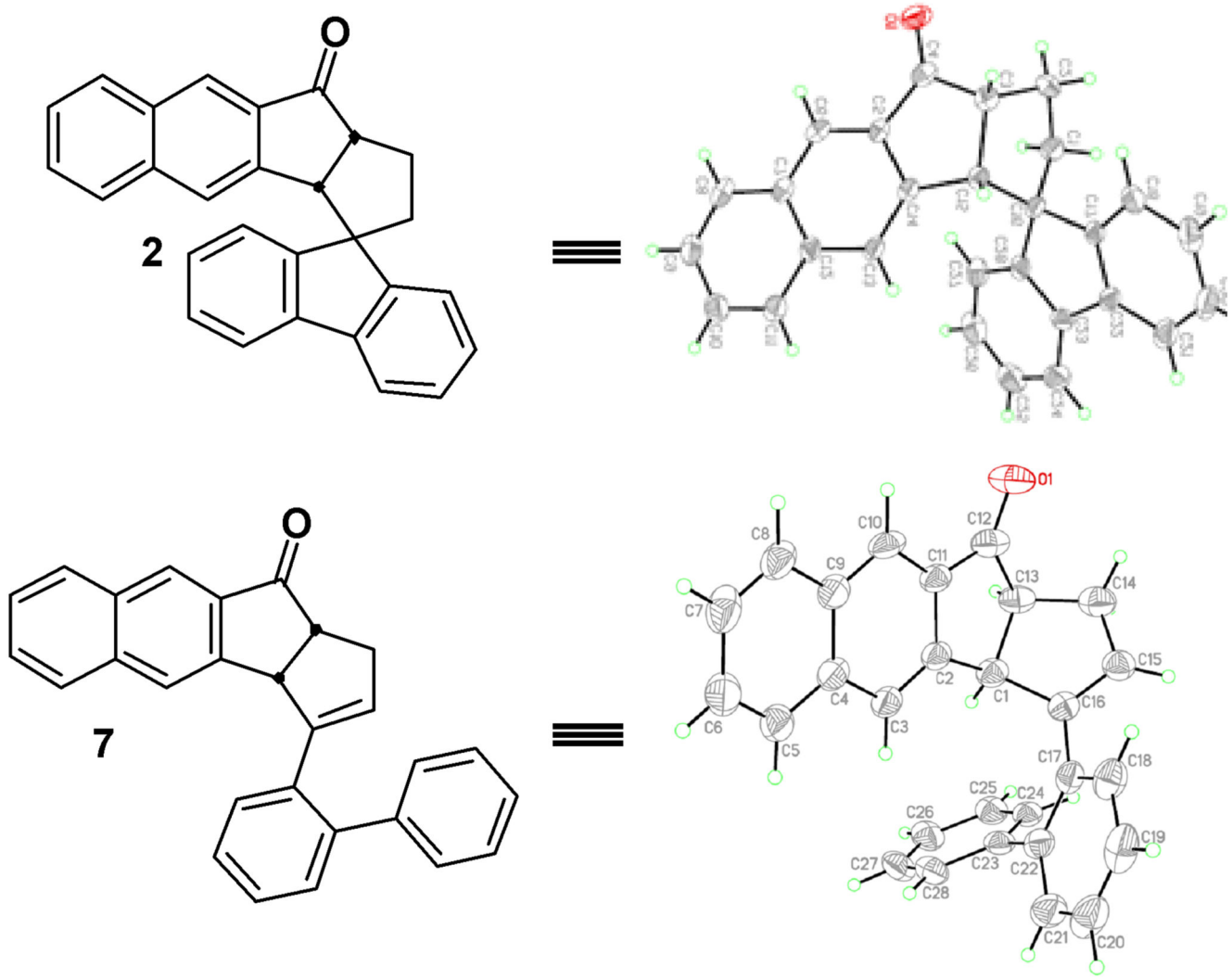



**Figure 2.**
X-ray structures of ketones **2** and **7**

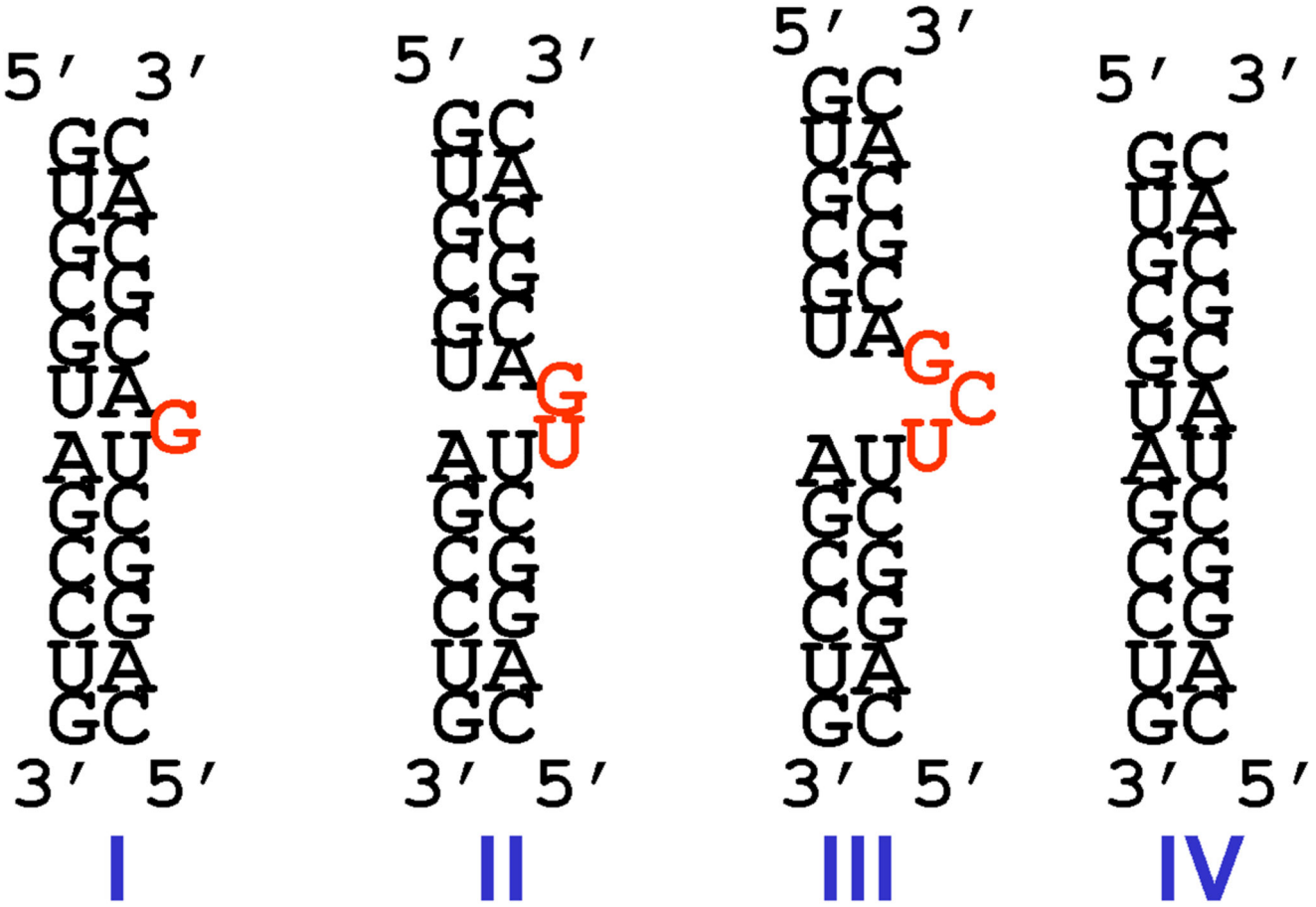
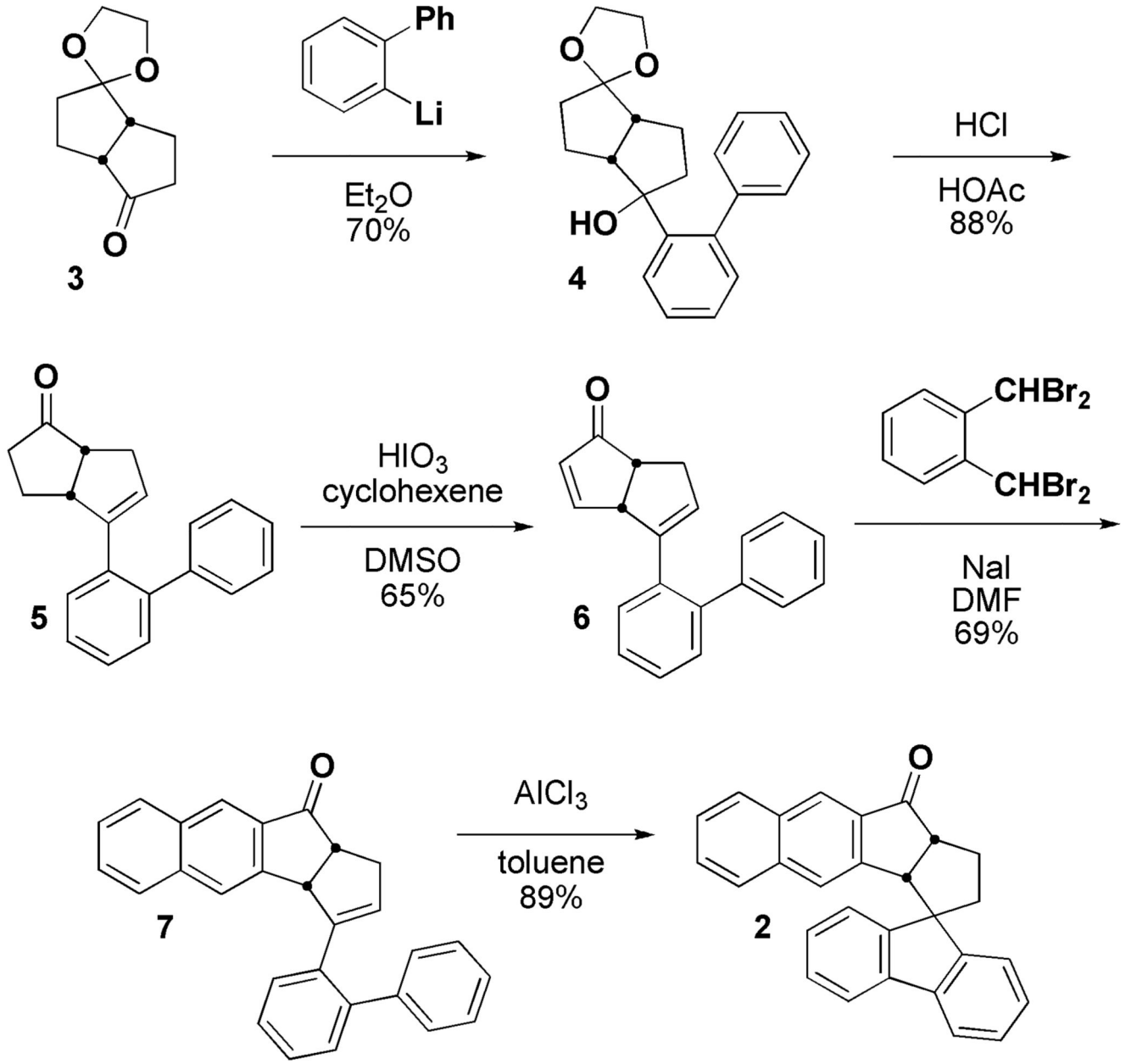
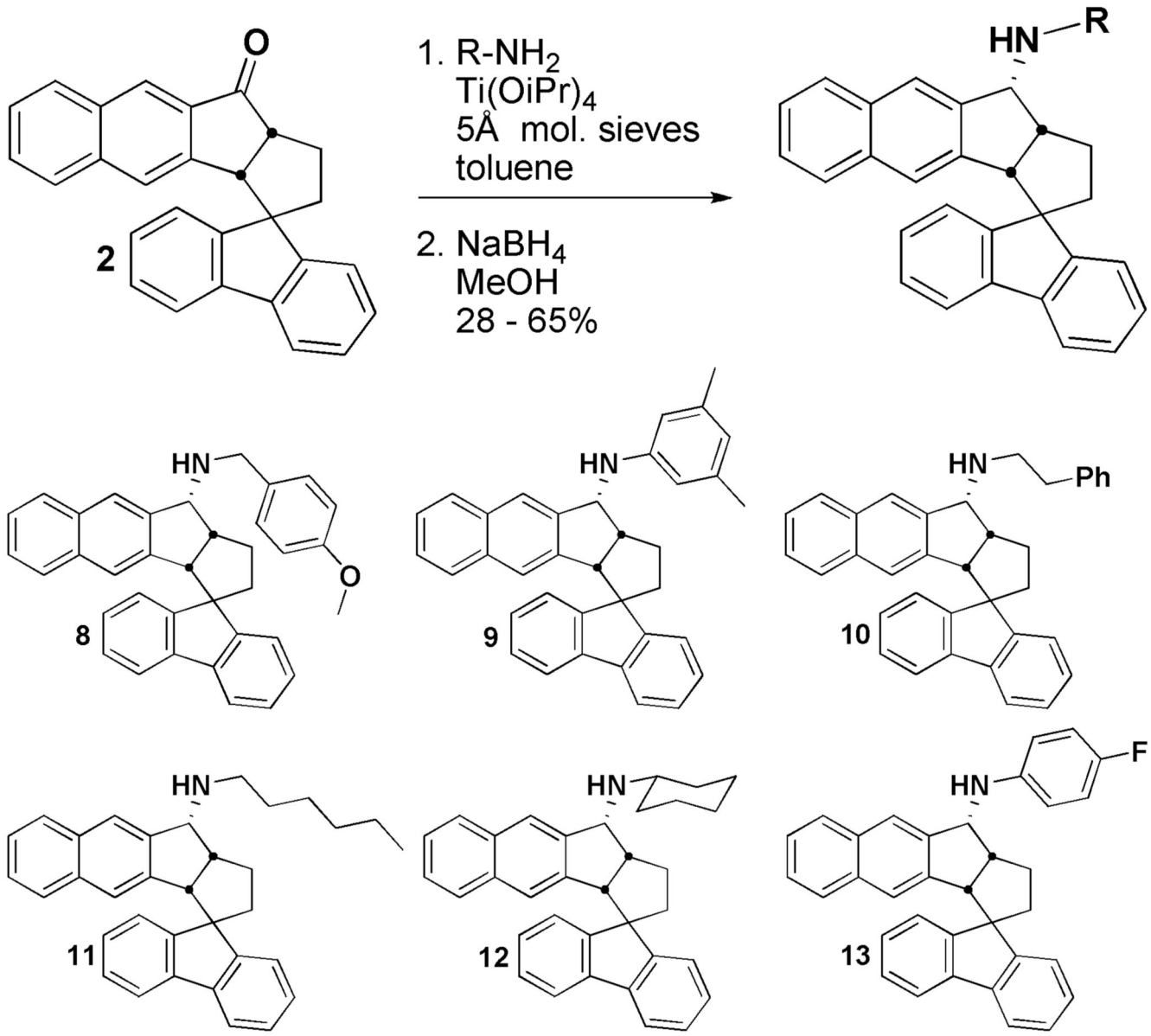


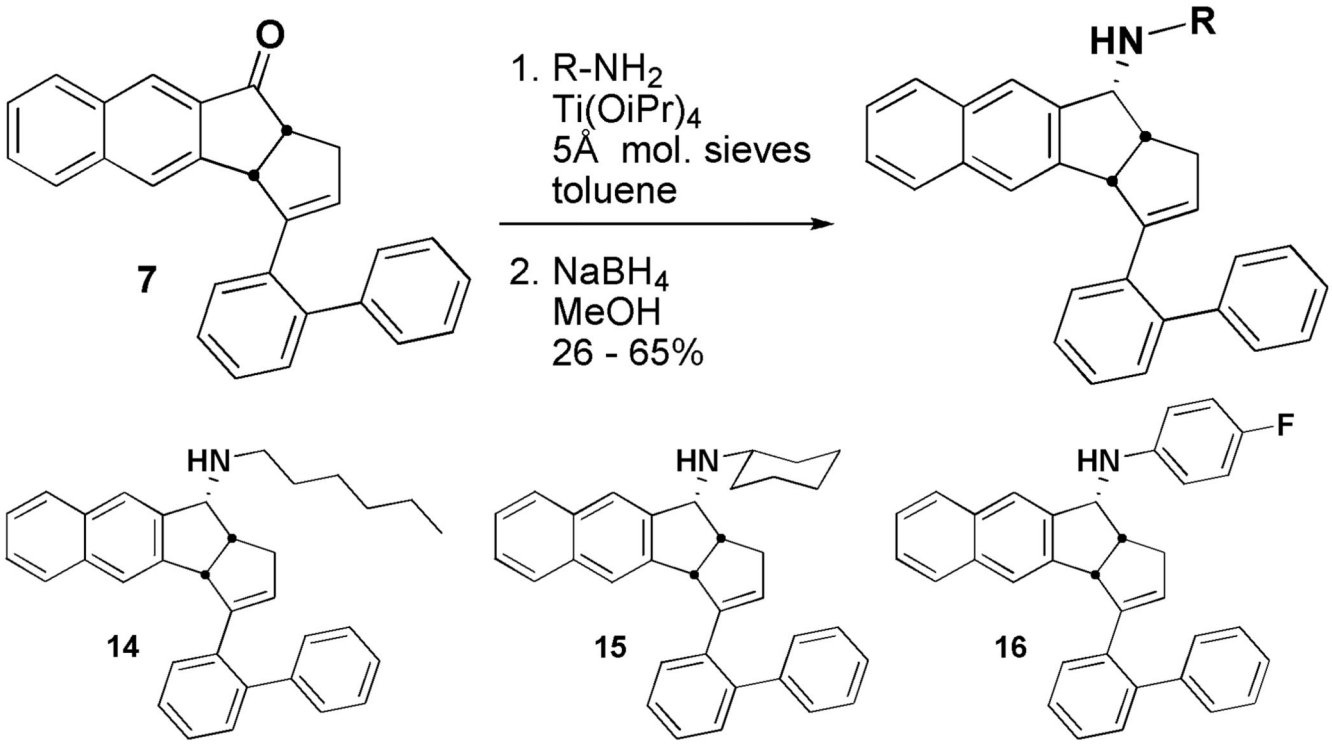

**Figure 3.**
Oligonucleotides for Binding Studies



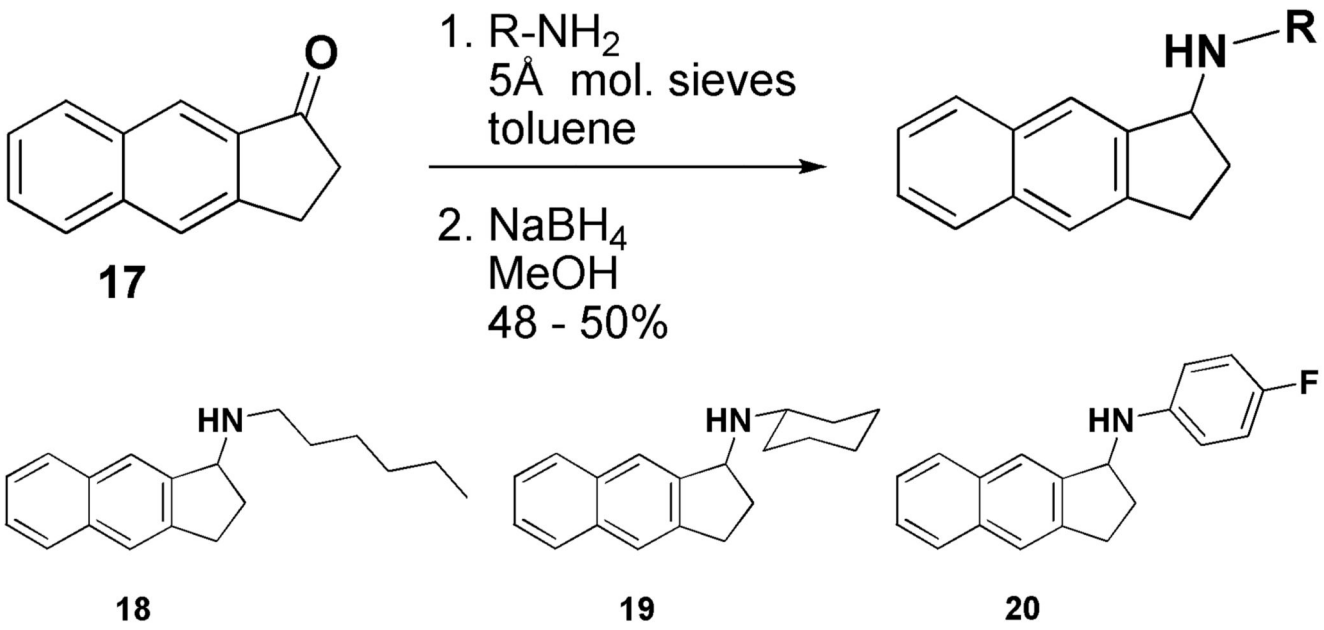

**Scheme 1.**
Synthesis of Spirocyclic Ketone **2**



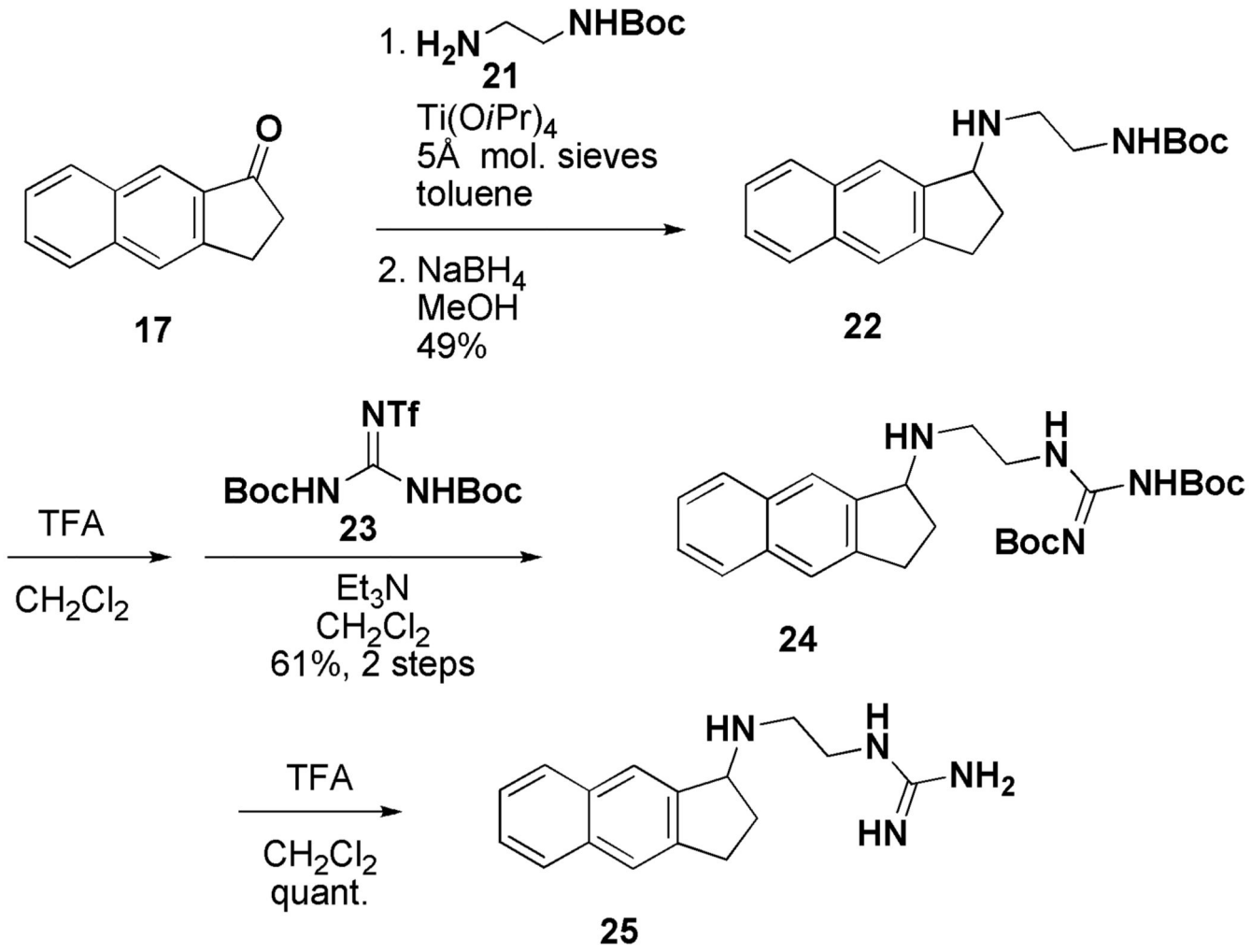

**Scheme 2.**
Synthesis of Spirocyclic Amines




**Scheme 3.**
Synthesis of Nonspirocyclic Amines




**Scheme 4.**
Synthesis of 2-aminobenzindanes




**Scheme 5.**
Synthesis of Guanidinylated Compound

**Table 1**

Binding Constants for RNA-Ligand Interactions[a]

| | I | II | III | IV |
|---|---|---|---|---|
| **2** | >20 | >20 | >20 | >20 |
| **7** | >20 | >20 | >20 | >20 |
| **17** | >20 | >20 | >20 | >20 |
| **8** | >20 | >20 | >20 | >20 |
| **9** | >20 | >20 | >20 | >20 |
| **10** | >20 | >20 | >20 | >20 |
| **11** | 12.1 | >20 | 6.1 | >20 |
| **12** | 9.7 | 5.9 | >20 | >20 |
| **13** | 7.8 | >20 | >20 | >20 |
| **14** | 12.4 | 9.4 | 11.5 | >20 |
| **15** | 6.4 | 2.3 | 18.1 | >20 |
| **16** | >20 | >20 | >20 | >20 |
| **18** | >20 | >20 | >20 | >20 |
| **19** | >20 | >20 | >20 | >20 |
| **20** | >20 | >20 | >20 | >20 |
| **25** | >20 | >20 | >20 | >20 |

[a]Binding constants in μM.