that recent theoretical studies[4b,c] have predicted the comparable stability of two model systems MeH_2Si^- and $(H_2N)H_2Si^-$, as well as their much higher stability than the corresponding carbanions.

We have now succeeded in the preparation of three stable (amino)(phenyl)silyl anions **1-3** by the standard direct reaction of aminochlorosilanes[13] **4-6** with lithium metal (Scheme I).[14] Thus, the reactions of **4** and **5** with lithium dispersion in THF set in at 0 °C to give immediately deep blue-green solutions; after 4 h, (aminosilyl)lithium **1** and (diaminosilyl)lithium **2** were formed in quantitative yields.[15] The reaction of **6** proceeded slowly to give **3** in 80% yield in 1 day at 0 °C. Significantly, the (aminosilyl)lithium **1** is stable at 0 °C for 6 days, and **2** and **3** are stable for 3 days without a drop in activity.

Functional silyl anions in solution are observable by ^{13}C and ^{29}Si NMR spectroscopy. Thus, aromatic carbons in $(Et_2N)Ph_2SiLi$ (**1**) in THF appear at 158.5 (ipso), 135.6 (ortho), 126.6 (meta), and 123.9 (para) ppm (cyclohexane δ 27.7 ppm as internal standard),[16] and chemical shift changes from the corresponding chlorosilane precursors $\Delta\delta$(SiLi - SiCl)[4j] are +24.3 (ipso), 0 (ortho), -21 (meta), and -7.3 (para) ppm (positive signs denote downfield shifts). The data are quite similar to those for $MePh_2SiLi$[4j] and imply that the Et_2N group exhibits essentially the same effect as the Me group on the charge distribution in the anions. The ^{29}Si resonance of **1** appears at -24.7 ppm in THF (TMS as external reference).

The versatility of the (aminosilyl)lithiums in organosilicon chemistry is apparent from some representative transformations shown in Scheme II.[17] All of the (aminosilyl)lithiums **1-3** undergo coupling with a variety of chlorosilanes to form the corresponding disilanes **7-12**: the Si-N bonds in the primary products can be converted into the Si-Cl bonds by mixture with an acyl chloride such as acetyl chloride, as exemplified by the transformations from **7** and **8** to **11** and **12**, respectively. A one-step introduction of two functional silyl groups into a dichlorosilane and a stepwise Si-Si bond elongation by sequential treatment of a chlorosilane with (aminosilyl)lithiums are exemplified by the formation of trisilanes **13** and **14**, respectively. It is noted that all of the functional disilanes and trisilanes prepared herein are structurally rather simple, but are new compounds barely accessible by conventional methods.

(Aminosilyl)lithiums are also useful reagents for organic synthesis. Thus, **1** or the corresponding copper reagent serves as the hydroxy anion equivalent through the conjugate addition to α,β-unsaturated esters or the allylic substitution followed by oxidative cleavage of the silicon-carbon bonds,[18] as shown in Scheme III.[17] The present procedure is complementary to the known $PhMe_2Si^-$ chemistry,[19] which requires an acid treatment prior to the oxidation. In particular, the latter method cannot be applied to allylic silane systems, because the acid treatment must cleave the particular allyl-silicon bond much faster than the phenyl-silicon bond.[20] The present aminosilyl anion chemistry has afforded a solution to this problem.

The present development, 40 years after the first practical synthesis of silyl anions,[5] has opened up the possibility of preparing a large variety of functional organosilicon compounds, including tailor-made polyfunctional disilanes, oligosilanes, and eventually polysilanes of current interest,[21] as well as new synthetic reagents for organic synthesis.

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Registry No. 1, 140438-35-3; **2**, 140438-36-4; **3**, 140438-37-5; **4**, 33935-31-8; **5**, 87651-58-9; **6**, 140438-38-6; **7**, 140438-39-7; **8**, 140438-40-0; **9**, 140438-41-1; **10**, 140438-42-2; **11**, 140438-43-3; **12**, 140438-44-4; **13**, 140438-45-5; **14**, 140438-46-6; $ClSiMe_2H$, 1066-35-9; $ClSiMe_2(NEt_2)$, 6026-02-4; $ClSiMe_2(O\text{-}i\text{-}Pr)$, 1825-71-4; $ClSiMe_2CH{=}CH_2$, 1719-58-0; Cl_2SiMe_2, 75-78-5; (*E*)-$CH_3CH{=}CHCO_2Et$, 623-70-1; (R*,S*)-$CH_3CH(SiPh_2(OEt))CH(CH_3)CO_2Et$, 140438-47-7; (R*,R*)-$CH_3CH(OH)CH(CH_3)CO_2Et$, 51898-36-3; $\overline{CH_2(CH_2)_4C}{=}CHCH_2SiPh_2(OEt)$, 140438-48-8; $\overline{CH_2(CH_2)_4C}{=}CHCH_2OH$, 932-89-8; 1-acetoxy-1-ethenylcyclohexane, 6318-49-6.

(13) Tamao, K.; Nakajo, E.; Ito, Y. *Tetrahedron* **1988**, *44*, 3997.

(14) Anions **1** and **2** were also obtained in 90% and 77% yields, respectively, by tin-lithium exchange reactions of (aminosilyl)stannanes $(Et_2N)_nPh_{3-n}SiSnMe_3$ with butyllithiums. Cf., Lipshutz, B. H.; Reuter, D. C.; Ellsworth, E. L. *J. Org. Chem.* **1989**, *54*, 4975 and references cited therein.

(15) Typically, under a nitrogen atmosphere, to a suspension of lithium dispersion (16 mg-atom: commercial 25 wt % in mineral oil was washed with dry hexane three times) in dry THF (8 mL) was added dropwise aminochlorosilane **4** (4 mmol) at room temperature. After a few minutes of stirring, the resulting greenish mixture was stirred at 0 °C for 4 h to give a solution of **1**. Lithium wire (fresh cuts) can also be used. Yields of the silyl anions were estimated by GLC analysis of the corresponding disilanes obtained by quenching with Me_3SiCl.

(16) Diaminosilyl anion **2** showed similar chemical shifts: 160.0 (ipso), 134.9 (ortho), 126.6 (meta), and 123.2 (para) ppm.

(17) Isolated yields are shown. All new compounds show satisfactory spectral and analytical data.

(18) (a) Tamao, K.; Ishida, N.; Tanaka, T.; Kumada, M. *Organometallics* **1983**, *2*, 1694. (b) Tamao, K.; Ishida, N.; Ito, Y.; Kumada, M. *Org. Synth.* **1990**, *69*, 96 and references cited therein.

(19) For example: (a) Fleming, I. *Pure Appl. Chem.* **1988**, *60*, 71. (b) Fleming, I.; Sanderson, P. E. J. *Tetrahedron Lett.* **1987**, *28*, 4229.

(20) (a) Fleming, I.; Dunogues, J.; Smithers, R. *Org. React.* **1989**, *37*, 57. (b) Magar, S. S.; Fuchs, P. L. *Tetrahedron Lett.* **1991**, *32*, 7513.

(21) Recent reviews: (a) West, R. *J. Organomet. Chem.* **1986**, *300*, 327. (b) West, R. In *The Chemistry of Organic Silicon Compounds*; Patai, S., Rappoport, Z., Eds.; Wiley: Chichester, England, 1989; pp 1208-1240. (c) Miller, R. D.; Michl, J. *Chem. Rev.* **1989**, *89*, 1359. (d) *Silicon-Based Polymer Science: A Comprehensive Resource*; Zeigler, J. M., Gordon Fearon, F. W., Eds.; American Chemical Society: Washington, DC, 1990.

Stereospecific Recognition of Tryptophan Agarose by in Vitro Selected RNA

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RNA molecules can recognize substrates by forming binding pockets and clefts similar to those found in proteins.[1] A better understanding of RNA-substrate recognition would facilitate the development of new ribozymes and receptor molecules based on RNA. Most protein enzymes bind substrates with enormous specificity, and in many cases the recognition is highly stereoselective. Here we show that this high degree of specificity can also be achieved by RNA.

The only well-characterized example of small-molecule recognition by a macromolecular RNA is the binding of guanosine (and its analogues, including arginine) by the self-splicing group I intron from *Tetrahymena*.[2] L-Arginine inhibits GTP binding and subsequent splicing of group I introns from several organisms with a 2-fold higher K_i than the D enantiomer,[3] corresponding to a $\Delta\Delta G$ of 0.4 kcal mol^{-1}. We have addressed the question of stereospecific substrate recognition by RNA by using in vitro selection[4] to isolate RNA molecules that are able to discriminate between D- and L-tryptophan coupled to an agarose matrix (D-tryptophan agarose, D-**Trp-A**). We have previously used in vitro selection to isolate RNAs and DNAs that specifically bind to small organic dye molecules from a large pool of random sequence RNA

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(1) (a) Latham, J. A.; Cech, T. R. *Science* **1989**, *245*, 276. (b) Celander, D. W.; Cech, T. R. *Biochemistry* **1990**, *29*, 1355.

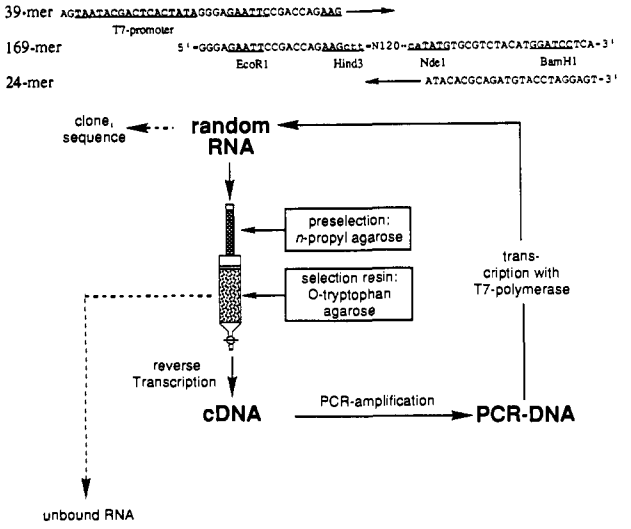
(2) (a) Bass, B. L.; Cech, T. R. *Nature* **1984**, *308*, 820. (b) Bass, B. L.; Cech, T. R. *Biochemistry* **1986**, *25*, 4473. (c) Michel, F.; Hanna, M.; Green, R.; Bartel, D. P.; Szostak, J. W. *Nature* **1989**, *342*, 391. (d) Yarus, M. *Science* **1988**, *240*, 1751-1758. (e) Von Ahsen, U.; Schroeder, R. *Nucleic Acids Res.* **1991**, *19*, 2261-2265.

(3) Hicke, B. J.; Christian, E. L.; Yarus, M. *EMBO J.* **1989**, *8*, 3843-3851.

(4) Famulok, M.; Szostak, J. W. *Angew. Chem.*, in press.

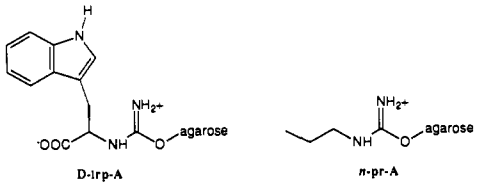
0002-7863/92/1514-3990$03.00/0

Scheme I. Design of the Random 169-mer Oligonucleotide and Scheme for the Selection of RNAs that Bind D-Trp-A[a]



[a] The underlined positions in the 169-mer indicate restriction sites. Bases in small letters represent defined positions that are not part of the primer binding sites.

Scheme II



or DNA molecules by the reiterated application of a cycle of purification by affinity chromatography followed by PCR amplification.[5] The process is outlined in Scheme I.

We designed and synthesized a 169-mer DNA oligonucleotide consisting of 120 random nucleotides flanked by two defined primer binding sites for PCR amplification. The 5'-primer contains a T7 promoter, so that RNA can be generated by in vitro transcription. Of the 700 μg of synthetic DNA, only 5% was free of chemical lesions and could be amplified by PCR, resulting in an effective pool complexity of $\sim 5 \times 10^{14}$ different sequences. After PCR amplification, the DNA was transcribed to yield a pool of ^{32}P-labeled RNA. This RNA was loaded onto a D-**Trp-A** column, and after washing with several column volumes of buffer, bound species were eluted with EDTA and reverse transcribed. The cDNA was amplified by PCR and transcribed to yield an enriched pool for subsequent rounds of selection. In order to remove RNA molecules that bind nonspecifically to the agarose matrix rather than to the substrate, the RNA pool was preselected on *n*-propylamine agarose (***n*-pr-A**) (Scheme II). The preselection column was washed with two column volumes of a buffer (4 mM Mg^{2+}, 250 mM NaCl, 50 mM Tris Cl, pH 7.6), and the eluate was applied directly to a D-**Trp-A** column.

In the first selection cycle, only 0.08% of the pool bound to the D-**Trp-A** column, but after seven cycles of selection and amplification 55% of the RNA bound to this column. The RNA pool from cycle 7 was amplified, and the binding specificity of the pool was tested on agarose derivatized (in the same way as the D-**Trp-A**) with a variety of different substrates (Table I). No significant binding to agarose derivatives other than the D-**Trp-A** was observed. The recognition of the D-**Trp-A** by the selected RNA species is stereoselective since the RNA does not bind to the diastereomeric L-**Trp-A**, even at a 9-fold higher substrate concentration. Considering that the selection procedure only required

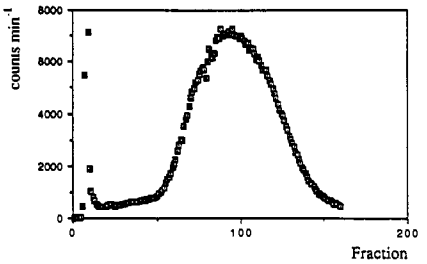
(5) (a) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818–822. (b) Ellington, A. D.; Szostak, J. W. *Nature* **1992**, *355*, 850–852.

Table I. Specificity of the D-Tryptophan Binding RNA Pool from Selection Cycle 7[a]

entry	substrate[b]	[substrate] (mM)	% binding
1	D-tryptophan	1.3	77
2	D-tryptophan	16.0	86
3	L-tryptophan	2.0	<4
4	L-tryptophan	12.0	<4
5	L-tyrosine	10.0	<4
6	NAD^+	2.9	<4
7	flavine mononucleotide	0.7	<4
8	biotin	2.0	<4

[a] % binding = (counts in EDTA elution)100(total counts)$^{-1}$. Binding buffer is 250 mM NaCl, 4 mM $MgCl_2$, 50 mM Tris, pH 7.6; elution buffer is 20 mM EDTA, pH 8.0. [b] The substrates in entries 1–7 were coupled to agarose through an isourea linkage (cyanogen bromide activation of the agarose). Substrate 8 was coupled by epoxy activation of the agarose.

Scheme III. Elution of MF-10 RNA from D-Trp-A[a]



[a] A 30-cm column was filled with 10.4 mL of D-**Trp-A** (27 cm, 1.3 mM D-Trp), the RNA was applied, and 1.45-mL fractions were collected. The initial peak at fraction 6 presumably represented unfolded RNA and was used to calculate the void volume of the column. The peak maximum with a maximum at fraction 92 represents RNA that has eluted after 13 column volumes due to D-**Trp-A** binding.

discrimination between D-**Trp-A** and ***n*-pr-A**, the specificity of the pool for D-**Trp-A** is remarkable and suggests that stereospecificity is an intrinsic property of substrate binding by RNA.

Sequencing of 10 clones from pool 7 revealed sequences with no detectable sequence similarity. Apparently, many different RNA structures are able to bind specifically to the substrate. We estimate the complexity of the pool to be 100–1000 different sequences.[6]

The dissociation constant for the binding of the clone MF-10[7] to D-**Trp-A** was determined by analytical affinity chromatography[8] and was found to be 18 μM (Scheme III). When applied to the 12.0 mM L-**Trp-A**, this RNA elutes almost in the void volume of the column. We calculate the K_D to be ≥12 mM, which corresponds to a $\Delta\Delta G$ of ≥3.9 kcal mol^{-1}.

In summary, this work demonstrates that, starting from a large pool of random sequence molecules, RNA sequences can be isolated that specifically recognize substrates that differ only by a single stereocenter. The relatively tight binding to D-**Trp-A** suggests that these RNAs have tertiary structures with binding pockets similar to those found in proteins.

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(6) The details on which this estimate is based will be published in the forthcoming full paper.

(7) This RNA contains the 114-nucleotide sequence 5'-TTGGC GTTGG CATGA CGCGG GGAAT CGGGT GCATC GATGA CTACT CCTGG GCCCA CGTCT GTTGT TGACG TCACA GCTTG ATTTA GGATA GCGCT TGGGC AGTCG TGCAG TGGA-3' between the 5'-39-mer and the 3'-24-mer primer binding sites (see Scheme I). The 3'-primer site is not required for binding: when this primer site was cut off with *Nde*I and the resulting DNA was transcribed, the RNA still bound specifically to D-**Trp-A** and not to L-**Trp-A**.

(8) (a) Arnold, F. H.; Schofield, S. A.; Blanch, H. W. *J. Chromatogr.* **1986**, *355*, 1–12. (b) Arnold, F. H.; Blanch, H. W. *Ibid.* **1986**, *355*, 13–27.