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Nucleotide Libraries as a Source of Biologically Relevant Chemical Diversity: Solution-phase Synthesis

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Abstract—Solution-phase parallel synthesis of nucleotide library **1** consisting of 150 members is reported.

Engineering diversity attributes that mimic the highly specific molecular recognition existing between nucleic acids, and that between proteins and nucleic acids, will be of great value in molecular discovery, particularly when compounds with biological activity are desired. It is anticipated that a nucleic acid-based (NAB) scaffold might be an appropriate template upon which such diversity can be built. There are at least three distinguishing features associated with NAB diversity: (a) as in the case of small molecules, a NAB scaffold can be used to create spatial diversity by variable display of hydrophobic, ionic, hydrogen bonding, and charge-transfer interactions, (b) a NAB scaffold can be engineered to create libraries that display 'shapes in space' (e.g., by incorporation of shape-defining motifs such as guanosine-quartets, bulges, stem-loops, circles, pseudoknots, etc.), and (c) by switching from deoxyribonucleoside to ribonucleosides to open-chain analogues, the furanose ring pucker can be changed from energetically favored $C_{2'}$-*endo* to $C_{3'}$-*endo* to extended conformations, thereby enabling overall changes in the local and/or global conformation of the library members. Such versatility in molecular engineering makes libraries modeled upon a NAB scaffold potentially a unique source of biologically relevant chemical diversity.[1,2]

In connection with our discovery program in antiviral therapeutics, we were interested in libraries of the general structure **1** (Fig. 1 and Table 1) in which the 5′-end of a nucleotide carried a variety of hydrophobic elements. Compounds in such libraries could potentially serve as inhibitors of viral kinases, viral polymerases, and/or as disrupters of helicase–primase complexes with nucleic acids during viral replication cycle. Specifically, in structure **1**, whereas the nucleotide element could provide the primary recognition element, the R group could provide hydrophobic interactions that could facilitate high affinity binding between the nucleotide and the target receptor. Furthermore, certain R groups could also aid in the intracellular delivery of the nucleotide. To the best of our knowledge, this concept has not been exploited in the context of nucleotide libraries. Interestingly, it was recently reported that the attachment of a hydrophobic steroidal moiety to the 5′-end of an oligonucleotide provided enhanced affinity of an oligonucleotide towards its complementary strand compared to the unmodified counterpart, the effect being attributed to productive van der Waals interactions between the steroid and the nucleotides.[3]

Tethering the 5′-end of a nucleoside with hydrophobic groups (R) each carrying additional functional groups can generate the library **1**. We have recently reported a solid-phase approach providing access to a subset of library **1**.[4] However, this approach has some limitations: (a) Restricted by resin-loading capacities, the solid-phase approach was in general not amenable to providing larger quantities of material for biological evaluation against multiple targets. (b) The need to use large excesses of reagents in solid-phase chemistry also affects the economics of library preparation, especially when the reagents are expensive. The herein reported library assembly by the solution-phase method[5] addresses some of these limitations. The main challenge in solution-phase combinatorial chemistry is the development of simple and efficient procedures to isolate/purify the intermediates and final products.[6] We report here a strategy to prepare a library of nucleotide analogues **1** using parallel solution-phase chemistry. In the event, simple liquid–liquid extraction

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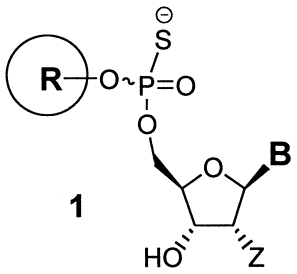
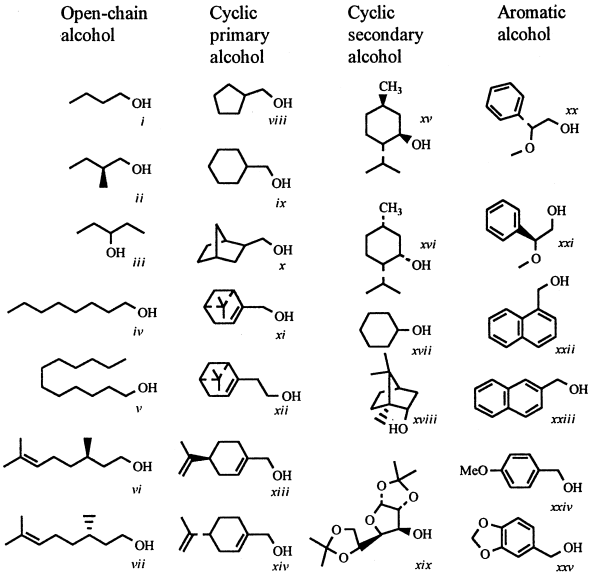
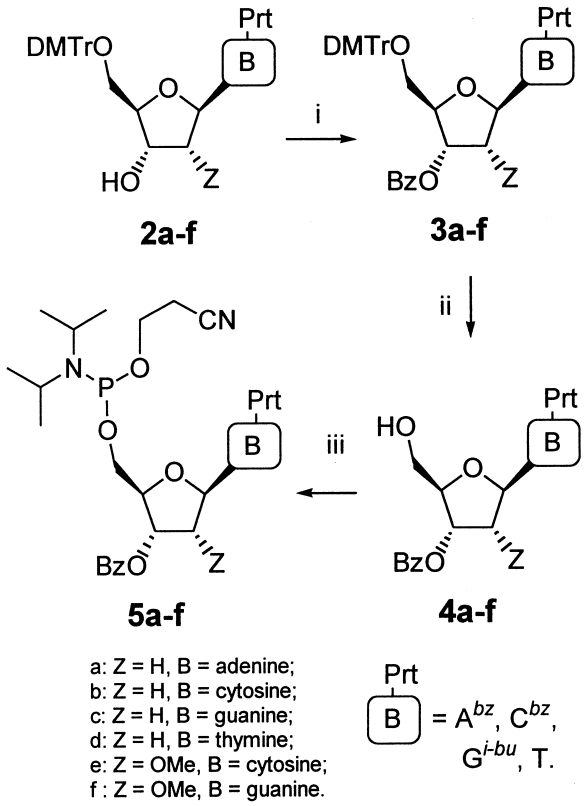


Figure 1. General structure of the library **1**. For description of R, B, and Z, see Scheme 1 and Table 1.

Table 1. Structures of alcohol entry **6** used in the synthesis of library **1**





Scheme 1. (i) BzCl/pyr; (ii) trifluoroacetic acid/$CHCl_3$; (iii) bis-(*N*,*N*-diisopropylamino)-2-cyanoethylphosphine, diisopropylammonium tetrazolide in CH_2Cl_2.

procedures have been tailored to isolate/purify both the key intermediates and final products. A prototype library comprised of 150 members has been assembled by this solution-phase method.

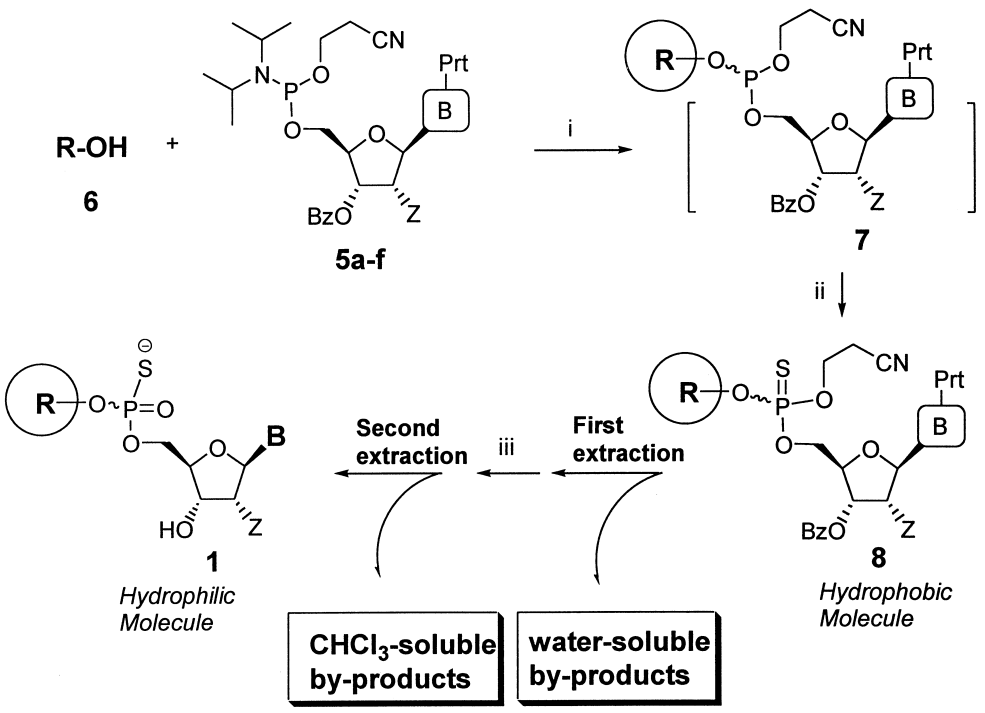
The requisite nucleoside building blocks were derived from deoxy- and ribonucleosides **2a**–**f** whereas the R groups were derived from 25 primary and secondary alcohols each with differing degrees of hydrophobicity, as well as, steric, and electronic properties (Table 1). The R groups were coupled to the nucleoside units via phosphorothioate linkage that (a) ensured the stability of the compound against nuclease-mediated degradation, (b) provided desirable ionic interaction of the compound with the target, and (c) imparted aqueous solubility to the compound.

Synthesis of Building Blocks

The synthesis of nucleoside building blocks **5a**–**f** were accomplished from **2a**–**f** via **3**, **4a**–**f** as shown in Scheme 1 (see also supporting information). The commercially available nucleosides **2a**–**f** were employed for the preparation of the 3′-benzoate derivatives **3a**–**f**. However, unmasking of the 5′-DMTr group in **3a**–**f** could not be readily effected using 3% dichloroacetic acid in dichloromethane, the commonly used "deblock" reagent. Alternatively, a short exposure to trifluoroacetic acid was very efficient in providing quantitative yields of **4a**–**f** (yield, 85 to 90% from **2a**–**f**), following which each nucleoside was converted to the corresponding phosphoramidite derivative **5a**–**f** by reported procedures (yield, 87 to 90% from **4a**–**f**).[7,8] The phosphoramidites **5a**–**f**, thus obtained, were characterized by NMR (^{1}H and ^{31}P) and HRMS.

Assembly of the Library

First, a model library was assembled (Scheme 2), using **5a**–**f** and a set of six alcohols. The reactions were done on a 20 to 30 μmol scale (see Experimental) in a parallel synthesis mode to obtain individual compounds. The key coupling reaction was initiated by the addition of 1*H*-tetrazole to a mixture consisting of each of the phosphoramidites **5a**–**f**, and the corresponding alcohol (Table 1). The resulting P(III) intermediates **7** were oxidatively sulfurized in situ by the addition of 3*H*-1,2-benzodithiole-3-one-1,1-dioxide (3*H*-BD) solution.[9] The presence of diisopropylammonium tetrazolide did not appear to impede the in situ sulfurization by 3*H*-BD, a compound otherwise known to be sensitive to various reagents.



Scheme 2. For description of Prt, B, Z see Scheme 1. The structures of alcohol entry **6** are depicted in Table 1. (i) 1*H*-Tetrazole in CH_3CN; (ii) 3*H*-1,2-benzodithiole-3-one-1,1-dioxide in CH_3CN; (iii) 28% NH_4OH.

The purification of the organic soluble triester intermediates **8** was achieved by partitioning between ethyl acetate and 5% sodium bicarbonate. It was anticipated that this process would facilitate the retention of the byproducts (diisopropyl ammonium tetrazolide and the sulfurization byproducts 1-benzothiole-2-oxa-3-one sulfoxides) in the aqueous phase. The R_p, S_p diastereomeric triesters **8** were then converted to product diesters **1** by treatment with 28% NH_4OH in quantitative yields. Following this step, the purification of the crude products was achieved by simple extraction of the aqueous layer with chloroform to remove the chloroform-soluble byproducts, while the product phosphorothioates **1** were retained in the aqueous layer. A final desalting step using C-18 cartridges (Sep-Pak® Plus, Waters) resulted in final products **1** of greater than 90% purity as determined by peak area in analytical HPLC (see Table 2).[4] The yields of the library members ranged from 75 to 85% starting from the amidites **5a–f**. Analysis by NMR and MS confirmed the structures of the products. Spectral analysis of selected members revealed that no detectable base-modifications had occurred during the synthesis.

Table 2. Spectral data and R_t values of representative compounds[a]

Compound	HPLC R_t (Min)	^{31}P (*ppm*)
1a-*i*	30.5	58.97, 58.64
1b-*i*	28.6	58.96
1c-*i*	28.1	58.91, 58.61
1d-*i*	29.7	58.32, 58.24
1a-*xix*	36.0	59.39, 59.33
1b-*xix*	35.1	59.75, 59.24
1c-*xix*	33.6	59.36, 59.21
1d-*xix*	38.2	59.72, 59.25
1e-*xix*	33.7	59.45, 59.18
1f-*xix*	32.5	59.30, 59.06
1a-*xx*	35.1	59.09, 58.73
1c-*xx*	32.6	59.06, 58.64
1b-*xii*	47.5	58.91, 58.67
1d-*xii*	48.2	58.97, 58.52
1e-*xii*	46.1	59.00, 58.68
1a-*xiii*	45.3	59.12, 58.81
1a-*xiv*	44.4	59.70, 59.41
1a-*xxii*	40.4	59.24, 59.13
1a-*xxiii*	41.1	59.70, 59.41

[a]For description of **a–f** see Scheme 1. The Roman numbers correspond to alcohols **6** listed in Table 1. For details of HPLC analysis, see ref 4.

Having established the conditions for the synthesis, a 150-member library **1** was assembled using 25 alcohols (Table 1) and six nucleoside building blocks (**5a–f**). The quality of the resulting library was evaluated by analytical HPLC and each member was found to have purity greater than 90% following a final desalting step.

Three significant features were recognized in terms of the power and facility of the herein reported solution-phase assembly of the library **1**: (a) the key triester intermediates **8** could be synthesized in a one-pot reaction system at room temperature in good yield by stepwise addition of reactants, (b) isolation/purification of the triesters **8** and final products **1** could be conveniently achieved by liquid–liquid extraction procedures, (c) the assembly method could be amenable to parallel combinatorial synthesis and 'split and pool' strategy[10] and could be automated.

The members of library **1** were evaluated as individual compounds (R_p, S_p diastereomeric mixture of phosphorothioates) for antiviral activity against hepatitis- and herpes-virus replication and a number of 'hits' have

been identified in cell-based assays.[11] The resulting structure–activity relationship is helping to guide the lead development through synthesis and evaluation of additional focused libraries. Details of these ongoing efforts will be reported in due course.

Experimental Procedure for Assembly of the Library

Each alcohol (30 μmol), and each of the nucleoside amidites **5a**–**f** (20 μmol) were added sequentially to a series of conical microtubes (2 mL, Ultident Scientific) containing 1*H*-tetrazole solution in acetonitrile (1 mL, 100 μmol). The mixture was shaken in a platform shaker at room temperature for 5 min. Then 3*H*-BD (40 μmol) was added as a solid, and the contents shaken for another 5 min. The acetonitrile was evaporated in a Speed Vac. Ethyl acetate (0.8 mL) was then added, followed by aqueous sodium bicarbonate (0.4 mL). Following thorough mixing of the phases, the organic layer containing the intermediate thiophosphate triester **8** was separated and evaporated to dryness. Aqueous ammonium hydroxide (28%, 1 mL) was added to the residue in each microtube. The tightly capped tubes were heated at 55 °C for 3 h. The aqueous ammoniacal solution was concentrated to dryness in a Speed Vac. The contents were dissolved in water (0.8 mL), and extracted with chloroform (2 × 0.4 mL). The aqueous layer was evaporated to dryness in a Speed Vac to obtain the library **1**. Each member was obtained as a white solid. Quantitation was achieved on the basis of A_{260} units, and the yields of product **1** were found to range from 75 to 85% starting from **5a**–**f**.

NMR and MS data of the representative library members: 1a-*i*. ^{1}H NMR (D_2O, 500 MHz): δ 8.44 (d, 1H, J = 9.2 Hz), 8.21 (s, 1H), 6.44–6.47 (m, 1H), 4.24 (s, 1H), 3.96–4.06 (m, 2H), 3.55–3.64 (m, 2H), 2.81–2.88 (m, 1H), 2.57–2.62 (m, 1H), 1.30–1.35 (m, 2H), 1.04–1.09 (m, 2H), 0.67 (t, 3H, J = 7.5 Hz, J = 7.3 Hz) ppm. ^{31}P NMR (D_2O, 200 MHz): δ 58.97, 58.64 ppm. ES–MS (negative mode): calcd for $C_{14}H_{21}N_5O_5PS$, 402 (M); found m/z, 402. **1b-*i***: ^{1}H NMR (D_2O, 500 MHz): δ 8.05 (d, 1H, J = 7.7 Hz), 6.24 (t, 1H, J = 6.6 Hz), 6.10 (d, 1H, J = 7.7 Hz), 4.50–4.53 (m, 1H), 4.19 (s, 1H), 4.03–4.12 (m, 2H), 3.84–3.88 (m, 2H), 2.40–2.45 (m, 1H), 2.24–2.29 (m, 1H), 1.51–1.57 (m, 2H), 1.25–1.33 (m, 2H), 0.83 (t, 3H, J = 7.5 Hz). ^{31}P NMR (D_2O, 200 Mz): δ 58.96. ES–MS (negative mode): calcd for $C_{13}H_{21}N_3O_6PS$, 378 (M); found m/z 378.

Acknowledgements

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References and Notes

1. For an excellent treatise on nucleic acids chemistry, see: Sanger, W. *Principles of Nucleic Acids Structure*; Springer-Verlag: New York, 1984.
2. For selected reviews on molecular diversity and combinatorial methods to drug discovery, see: (a) Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Chem. Rev.* **1997**, 97, 449. (b) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233. (c) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385. (d) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555.
3. Bleczinski, C. F.; Richert, C. *J. Am. Chem. Soc.* **1999**, *121*, 10889.
4. Zhou, W.; Roland, A.; Jin, Y.; Iyer, R. P. *Tetrahedron Lett.* **2000**, *41*, 441.
5. For selected methods of library assembly using solution-phase approach, see: (a) Boger, D. L.; Tarby, C. M.; Myers, P. L.; Caporale, L. H.; *J. Am. Chem. Soc.* **1996**, 118, 2109. (b) An, H.; Haly, B. D.; Fraser, A. S.; Guinosso, C. J.; Cook, P. D. *J. Org. Chem.* **1997**, *62*, 5156. (c) Cheng, S.; Comer, D. D.; Williams, J. P.; Myers, P. I., Boger, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 2567. (d) Carell, T.; Wintner, E. A.; Bashir-Hashemi, A.; Rebek Jr, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2059.
6. Gayo, L. M. *Biotechnol. Bioeng.* **1998**, *61*, 95.
7. Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. *Nucl. Acids Res.* **1984**, *12*, 4051.
8. For methods of preparation of phosphoramidite derivatives, see: Iyer, R. P.; Beaucage, S. L. In *Comprehensive Natural Products Chemistry*; Kool, E. T., Ed.; Elsevier Science: London, 1999; Vol. 7, pp 105–152.
9. Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1253.
10. For a selected review, see: Houghten, R. A.; Pinilla, C.; Appel, J. R.; Blondelle, S. E.; Dooley, C. T.; Eichler, J.; Nefzi, A.; Ostresh, J. M. *J. Med. Chem.* **1999**, *42*, 3743.
11. The cell-based assays helped to validate the biological relevance of the libraries in terms of having 'drug-like' attributes. The antiviral screen results will be reported in a separate account in due course.