J|A|C|S COMMUNICATIONS

Published on Web 09/08/2010

High-Throughput Screening of Dendrimer-Binding Drugs

Libo Zhao,[†,‡] Qinglin Wu,[†,§] Yiyun Cheng,*[,§] Jiahai Zhang,[∥] Jihui Wu,[∥] and Tongwen Xu*[,‡]

CAS Key Laboratory of Soft Matter Chemistry, School of Chemistry and Material Science, University of Science and Technology of China, Hefei, Anhui 230026, People's Republic of China, School of Life Sciences, East China Normal University, Shanghai 200062, People's Republic of China, and Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230027, People's Republic of China

Received July 11, 2010; E-mail: yycheng@mail.ustc.edu.cn; twxu@ustc.edu.cn

Abstract: A convenient approach for the high-throughput screening of dendrimer-binding drugs by NMR techniques including saturation transfer difference (STD) NMR and Hadamard-encoded nuclear Overhauser effect measurements is presented. The screening results for insoluble drugs show that phenylbutazone and sulfamethoxazole prefer to localize in the interior pockets of dendrimer, while mycophenolic acid mostly binds on the dendrimer surface, and noncharged insoluble drugs like trimethoprim and primidone do not interact with dendrimers. In another path for soluble drugs, *n*-butanoic acid and dimethylformamide are screened as dendrimer-binding compounds from a screening pool containing eight soluble compounds by STD NMR. The screening of dendrimer-binding insoluble or soluble compounds can be finished within an hour.

High-throughput screening (HTS) is an essential approach to drug discovery that has revolutionized the pharmaceutical industry in the past decade.[1] It identifies the binding of a large number of biologically active compounds to a target of interest to facilitate the fast discovery of new chemical entities.[2] Today, dendrimer-based drug delivery systems have become more and more important in the pharmaceutical sciences.[3] Lots of drugs were reported to benefit from the formulation of dendrimer−drug complexes mediated by ionic/hydrogen-bond binding or hydrophobic encapsulation.[4] There is a potential for significant fragmentation of the literature if the physicochemical properties of dendrimer−drug complexes are published in a "one drug at a time" fashion. The greater availability of drugs in the libraries and the growth numbers of functional dendrimers have made it difficult for a pharmaceutist to design the most effective dendrimer−drug formulations in a relatively short time. In addition, the size/surface charge-tunable properties of dendrimers make them excellent candidates in the biomimicry of proteins or protein/DNA complexes.[3b] Screening of potential ligands for these "artificial proteins" is essential for the methodology of HTS in molecular recognitions between drugs and an interested biological target. Here we describe the use of NMR techniques including normal nuclear Overhauser effect (NOE) and Hadamard-encoded NOE measurements and saturation transfer difference (STD) NMR for the fast screening of dendrimer-binding drugs, which delivers meaningful hits for the optimization of dendrimer-based drug delivery systems.

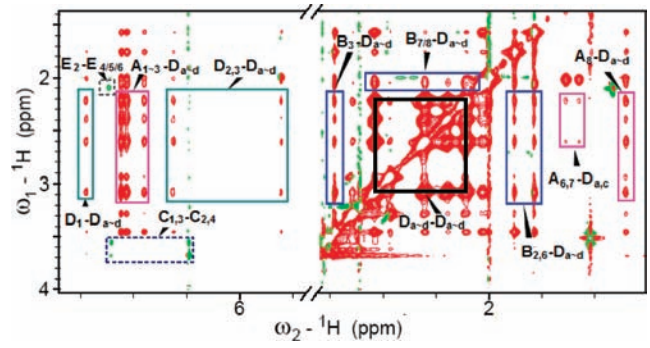


Figure 1. ^{1}H−^{1}H NOESY spectrum of dendrimer/insoluble drug complexes at a mixing time of 300 ms. Chemical shifts of drugs are assigned from TOCSY results shown in Figure S2 (A, phenylbutazone; B, mycophenolic acid; C, trimethoprim; D, sulfamethoxazole; E, primidone; EA, ethanol).

Chart 1. Molecular Structures of the Five Insoluble Drugs

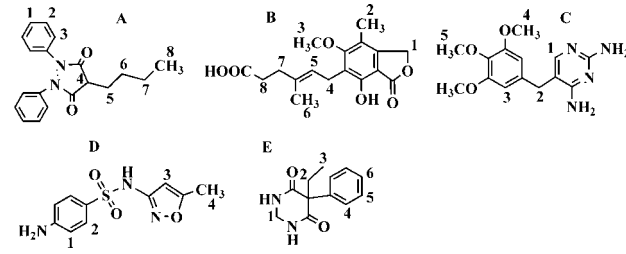


Figure 1 shows the transferred NOE (trNOE) spectroscopy of the dendrimer/insoluble drug complexes (phenylbutazone, mycophenolic acid, trimethoprim, sulfamethoxazole, and primidone, Chart 1 and Figure S1, Supporting Information) with a short mixing time of 300 ms. Strong trNOE cross-peaks are observed between scaffold protons of dendrimer (Ha−Hd) and aromatic protons of phenylbutazone and sulfamethoxazole, and medium trNOE signals between methylene protons of dendrimer (Ha, Hc, and Hd) and methyl protons of the phenylbutazone are observed, while weak trNOE interactions are observed between dendrimer and mycophenolic acid. However, no trNOE interaction between trimethoprim/primidone and dendrimer is found in Figure 1. It is well-known that the trNOE intensity of a cross-peak in NOE spectroscopy is proportional to the number of related protons and the distance between them.[5,6] The presence of trNOE signals between interior pockets of dendrimer and drug molecules suggests that these drug molecules are localized in the interior pockets.[7] Since trimethoprim and primidone are noncharged drug molecules and the other drugs in the screening pool are negatively charged,[4a] the trNOE results indicate that the formation of molecular inclusion in cationic

[‡] School of Chemistry and Material Science, University of Science and Technology of China.
[§] School of Life Sciences, East China Normal University.
[∥] Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China.
[†] These authors contributed equally to the manuscript.

10.1021/ja106128u

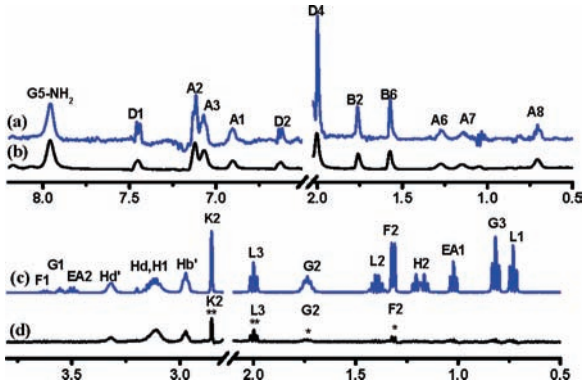
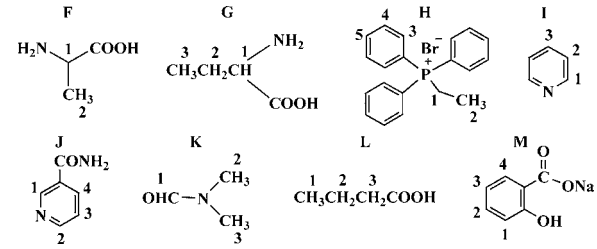


Figure 2. (a) Hadamard-encoded NOE and (b) normal NOESY of the dendrimer/insoluble drug complexes at mixing time of 300 ms. (c) ^{1}H NMR and (d) STD spectra of dendrimer/soluble compound complexes at high-field region. The chemical shift assignments of the insoluble and soluble compounds in the screening pool are assigned from the TOCSY results shown in Figure S2 and Figure S8, respectively (F, L-alanine; G, DL-2-aminobutyric acid; H, ethyl triphenyl phosphonium bromide; I, pyridine; J, nicotinamide; K, *N*,*N*-dimethylformamide; L, *n*-butanoic acid; M, sodium salicylate).

PAMAM dendrimer is charge-dependent and that electrostatic interaction between the guest and the cationic dendrimer is the first step of encapsulation. On the other hand, macromolecules exhibit negative NOE effect with a fast buildup rate, while the low-molecular-weight ligands (<1000 Da) have positive NOE signals with a slow buildup rate. If the low-molecular-weight ligand binds to the macromolecular receptor, it obtains motional correction time of the receptor, thus developing a negative NOE in the bound state.[7a,8a] The negative intramolecular trNOE signals (red) of the drugs in Figure 1 and Figure S3 indicate that phenylbutazone, sulfamethoxazole, and mycophenolic acid are in the bound state. The positive intramolecular cross-peaks (green) for primidone and trimethoprim reveal that most of these drugs are in the free state. Previous studies have reported that the solubilization factor of mycophenolic acid is much higher than that of sulfamethoxazole and phenylbutazone by dendrimer.[8b] The weak NOE signals between mycophenolic acid and dendrimers suggest that most of the guests were bound on the surface by ionic or hydrogen-bond interactions (Figure S11). These results confirm that trNOE is useful in the fast screening of dendrimer-binding ligands, giving information on molecular encapsulation/ionic binding, spatial distance, and molecular orientation in the host−guest systems.

To further reduce the experimental time in 2D trNOE experiments, we use the Hadamard-encoded cross-relaxation spectroscopy to speed up the HTS of dendrimer-binding drug molecules. The Hadamard-encoded NOESY measures NOE interactions for selected proton frequencies of dendrimer or drugs.[9] Figure 2a and Figure S4 show the NOE interactions between the protons in dendrimer and insoluble drugs from the Hadamard-encoded NOE experiment. The results are consistent with that obtained from a normal NOESY experiment in Figure 2b and Figure S5. To validate the screening results from NOESY and Hadamard-encoded NOE studies, we conducted NOE experiments for G5 dendrimer and single guest in the screening pool in Chart 1. As demonstrated in Figure S6, G5 dendrimer interacts with phenylbutazone, mycophenolic acid, and sulfamethoxazole but does not bind trimethoprim and primidone, which are fully consistent with the results obtained from the mixing pool. The Hadamard-assisted NOE spectrum provides the same information with reduced experimental time from whole days to several minutes, which is essential in the HTS of dendrimer-binding guests.

Chart 2. Molecular Structures of the Eight Soluble Drugs



For a screening pool containing eight water-soluble compounds (Chart 2), no obvious cross-peaks between dendrimer and the guests can be observed in the NOESY spectrum in Figure S7, suggesting that the dendrimers have a low tendency to encapsulate hydrophilic drug molecules. In this case, we use STD NMR to analyze the competitive binding of these compounds on the surface of dendrimers. STD NMR, which has been proven to be a sensitive and robust tool to study complexes involving receptor/ligand recognitions and host−guest interactions, is able to screen multiple compounds with relatively high binding affinity toward dendrimer simultaneously.[10] The ^{1}H NMR and STD NMR spectra of a G5 dendrimer solution with eight soluble compounds are shown in Figure 2c,d, respectively. The presence of strong STD signals for *n*-butanoic acid and *N*,*N*-dimethylformamide and medium STD signals for sodium salicylate, L-alanine, and DL-2-aminobutyric acid in Figure 2d and Figure S9 proves that these compounds in the solution bind to dendrimer. No STD signal for pyridine, ethyl triphenyl phosphonium bromide, and nicotinamide is observed, suggesting weak binding affinity of these compounds toward dendrimer. We also determined the binding behaviors of G5 dendrimer toward the soluble guests in Chart 2 without *N*,*N*-dimethylformamide and *n*-butanoic acid by STD NMR. As shown in Figure S10, the presence of strong STD signals for L-alanine and DL-2-aminobutyric acid indicates that a competitive binding occurs between G5 dendrimer and the ligands in the screening pool and that dendrimer is capable of binding drugs with weaker binding affinities. Compounds including *n*-butanoic acid, sodium salicylate, L-alanine, and DL-2-aminobutyric acid have negative charges in their structures which are responsive for the ionic interactions between dendrimer and the compounds. Strong binding affinity between *N*,*N*-dimethylformamide and dendrimers is due to the presence of a strong hydrogen-bond donor in the molecule which mediates hydrogen-bond interactions with dendrimer. The highest binding affinity of *n*-butanoic acid to the dendrimer among sodium salicylate, L-alanine, *n*-butanoic acid, and DL-2-aminobutyric acid is due to weak steric hindrance for the electrostatic attachment of *n*-butanoic acid on dendrimer surface.

In summary, we demonstrated the use of NMR techniques for HTS of dendrimer-binding guests. The whole HTS experiment for insoluble/soluble compounds by a combination of Hadamard-encoded NOE and STD NMR can be finished within an hour. To the best of our knowledge, this is the first report of screening dendrimer-binding guest molecules. The acquired NMR data also give information on the localization of guests, i.e., in the interior cavities or on the surface of dendrimers. The hydrophobic encapsulation of guest molecules in the interior means slower release rate which is expected in a sustained drug delivery system. The attachment of soluble guests on the surface of dendrimer via hydrogen-bond and ionic interactions leads to a much faster release rate in comparison with the encapsulations, and the formulations are important in the transdermal delivery of hydrophilic compounds to penetrate through the skin barrier consisted of phospholipids and

membrane proteins. It offers several features for the physicochemical properties of dendrimer-based host−guest systems and the design of dendrimer-based drug formulations. The findings obtained in this study can help with the generation of a large family of guest molecules which may benefit from the dendrimer inclusion/binding technique. Also, the methodology developed for the dendrimer system is essential for fast discovery of new host−guest systems and molecular-recognition processes.

Acknowledgment. We thank the Talent Program of East China Normal University (No.77202201) and the Specialized Research Fund for the Doctoral Program of Higher Education (No.20093402110041) for financial support to this program.

Supporting Information Available: Experimental procedures and further information on NMR-assisted high-throughput screening of dendrimer-binding drugs. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bista, M.; Kowalska, K.; Janczyk, W.; Domling, A.; Holak, T. A. *J. Am. Chem. Soc.* **2009**, *131*, 7500.

(2) Veldkamp, C. T.; Ziarek, J. J.; Peterson, F. C.; Chen, Y.; Volkman, B. F. *J. Am. Chem. Soc.* **2010**, *132*, 7242.

(3) (a) Astruc, D.; Boisselier, E.; Ornelas, C. *Chem. Rev.* **2010**, *110*, 1857. (b) Menjoge, A. R.; Kannan, R. M.; Tomalia, D. A. *Drug Discov. Today* **2010**, *15*, 171. (c) Medina, S. H.; EI-Sayed, M. E. *Chem. Rev.* **2009**, *109*, 3141. (d) Goodwin, A. P.; Lam, S. S.; Frechet, J. M. J. *J. Am. Chem. Soc.* **2007**, *129*, 6994. (e) Gillies, E. R.; Jonsson, T. B.; Frechet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 11936. (f) Lee, C. C.; Mackay, J. A.; Frechet, J. M. J.; Szoka, F. C. *Nat. Biotechnol.* **2005**, *23*, 1517. (g) Frechet, J. M. J. *J. Polym. Sci., Polym. Chem.* **2003**, *41*, 3713. (h) Lee, C. C.; Gillies, E. R.; Fox, M. E.; Guillaudeu, S. J.; Frechet, J. M. J.; Dy, E. E.; Szoka, F. C. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 16649.

(4) (a) Cheng, Y. Y.; Wu, Q. L.; Li, Y. W.; Xu, T. W. *J. Phys. Chem. B* **2008**, *112*, 8884. (b) Liu, M. J.; Frechet, J. M. J. *Pharm. Sci. Technol. Today* **1999**, *2*, 393. (c) Frechet, J. M. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4782.

(5) (a) Hu, J. J.; Cheng, Y. Y.; Ma, Y. R.; Wu, Q. L.; Xu, T. W. *J. Phys. Chem. B* **2009**, *113*, 64. (b) Hu, J. J.; Cheng, Y. Y.; Wu, Q. L.; Zhao, L. B.; Xu, T. W. *J. Phys. Chem. B* **2009**, *113*, 10650. (c) Hu, J. J.; Fang, M.; Cheng, Y. Y.; Zhang, J. H.; Wu, Q. L.; Xu, T. W. *J. Phys. Chem. B* **2010**, *114*, 7148.

(6) (a) Chai, M. H.; Niu, Y. H.; Youngs, W. J.; Rinaldi, P. L. *J. Am. Chem. Soc.* **2001**, *123*, 4670. (b) Chai, M. H.; Niu, Y. H.; Youngs, W. J.; Rinaldi, P. L. *Macromolecules* **2000**, *33*, 5395. (c) Chai, M. H.; Holley, A. K.; Kruskamp, M. *Chem. Commun.* **2007**, 168.

(7) (a) Smith, P. E.; Brender, J. R.; Durr, U. H.; Xu, J.; Mullen, D. G.; Banaszak Holl, M. M.; Ramamoorthy, A. *J. Am. Chem. Soc.* **2010**, *132*, 8087. (b) Cheng, Y. Y.; Wu, Q. L.; Li, Y. W.; Hu, J. J.; Xu, T. W. *J. Phys. Chem. B* **2009**, *113*, 8339. (c) Cheng, Y. Y.; Li, Y. W.; Wu, Q. L.; Xu, T. W. *J. Phys. Chem. B* **2008**, *112*, 12674. (d) Yang, K.; Cheng, Y. Y.; Feng, X. Y.; Zhang, J. H.; Wu, Q. L.; Xu, T. W. *J. Phys. Chem. B* **2010**, *114*, 7265. (e) Fang, M.; Cheng, Y. Y.; Zhang, J. H.; Wu, Q. L.; Hu, J. J.; Zhao, L. B.; Xu, T. W. *J. Phys. Chem. B* **2010**, *114*, 6048. (f) Wu, Q. L.; Cheng, Y. Y.; Hu, J. J.; Zhao, L. B.; Xu, T. W. *J. Phys. Chem. B* **2009**, *113*, 12934.

(8) (a) Handel, H.; Gesele, E.; Gottschall, K.; Albert, K. *Angew. Chem., Int. Ed.* **2003**, *42*, 438. (b) Zhao, L. B.; Cheng, Y. Y.; Hu, J. J.; Wu, Q. L.; Xu, T. W. *J. Phys. Chem. B* **2009**, *113*, 14172.

(9) (a) Kupce, E.; Freeman, R. *J. Magn. Reson.* **2003**, *162*, 300. (b) Feliz, M.; Garcia, J.; Aragon, E.; Pons, M. *J. Am. Chem. Soc.* **2006**, *128*, 7146. (c) Steinbeck, C. A.; Chmelka, B. F. *J. Am. Chem. Soc.* **2005**, *127*, 11624.

(10) (a) Szczygiel, A.; Timmermans, L.; Fritzinger, B.; Martins, J. C. *J. Am. Chem. Soc.* **2009**, *131*, 17756. (b) Streiff, J. H.; Juranic, N. O.; Macura, S. I.; Warner, D. O.; Jones, K. A.; Perkins, W. J. *Mol. Pharmacol.* **2004**, *66*, 929. (c) McCoy, M. A.; Senior, M. M.; Wyss, D. F. *J. Am. Chem. Soc.* **2005**, *127*, 7978.

JA106128U