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## Teaching old indicators even more tricks: binding affinity measurements with the guest-displacement assay (GDA)†

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A simple change has important consequences: the guest-displacement assay (GDA) is introduced which allows for binding affinity determinations of supramolecular complexes with spectroscopically silent hosts and guests. GDA is complementary to indicator-displacement assay for affinity measurements with soluble components, but is superior for insoluble or for weakly binding guests.

The physico-chemical description of supramolecular systems, *e.g.* host–guest complexes, usually focuses on the thermodynamic properties, *i.e.* binding affinities ( $K_a$ ) in an effort to develop binding models and to derive predictive structure–property correlations.<sup>1–11</sup>  $K_a$  values of guest-inclusion complexes have been reported for many different classes of synthetic hosts.<sup>12,13</sup> Also, protein–ligand binding can be described through a host–guest binding formalism.<sup>14</sup> There are two major classes of methods available to determine binding affinities of host–guest complexes, (i) direct-binding assays, and (ii) competitive-binding assays. In direct-binding assays (DBAs), the change in a spectroscopic property (*e.g.* NMR shift, absorbance, emission) or the heat of the reaction is recorded to construct a binding isotherm.<sup>3,15</sup> In each case, either a guest solution is titrated to a solution of the host or *vice versa*. Therefore, host and guest need to be soluble, which limits the number of host–guest combinations that can be compared in the same neat solvents. Similarly, in an indicator-displacement assay (IDA),<sup>15–17</sup> which is the most utilized competitive method for  $K_a$  value determination of supramolecular systems, a solution of a guest is titrated to a solution of a host-indicator complex (Fig. 1a). IDA is superior over DBA for obtaining affinities of spectroscopically silent host–guest complexes, but it is restricted to soluble guests.

A common workaround for the solubility limitations is the use of (complex) solvent mixtures, consisting of polar and apolar solvents in a suitable ratio to dissolve both the host and the guest.<sup>18</sup> Other additives such as salts can also be useful

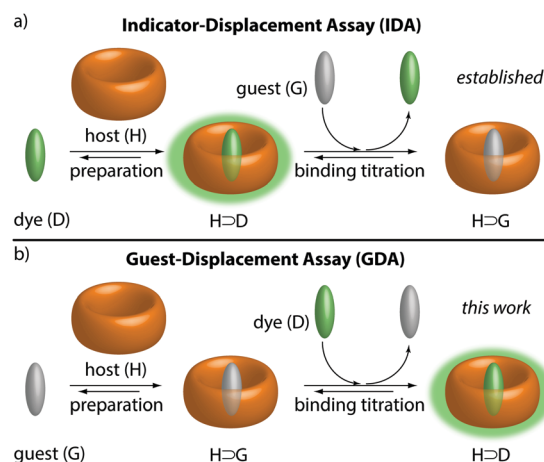


Fig. 1 Competitive binding assays for  $K_a$  value determination of spectroscopically silent host–guest complexes, (a) the indicator-displacement assay (IDA), (b) the herein introduced guest-displacement assay (GDA). GDA is generally complementary to IDA but superior for insoluble guests that can be solubilized as their H>G complexes, and for weakly binding guests that are readily displaced by the indicator dye.

to dissolve hosts or guests.<sup>19</sup> Nevertheless, this practically-motivated approach can cause fundamental problems; (i) even miscible solvents, *e.g.* water and methanol, may not mix on a molecular level,<sup>20</sup> such that preferential solvation of the host or guest by one solvent component may occur.<sup>21</sup> (ii) Competitive binding of solvent or salts to the host can result in apparent binding affinities of the host–guest complex of interest.<sup>19,22</sup> Such effects can occur even at low volume percentages of the cosolvent.<sup>23</sup>

As a result of the solvophobic effect, the most insoluble guests typically display the strongest binding affinities for supramolecular hosts,<sup>4,5,10,24,25</sup> but such compounds escape the accurate assessment of their  $K_a$  values with established methods.

In this contribution, we demonstrate an alternative approach for determining binding affinities of host–guest complexes which is now applicable to insoluble or weakly binding guests.

Cucurbit[ $n$ ]urils (CB $n$ )<sup>26–28</sup> and cyclodextrins (CD),<sup>29,30</sup> see Fig. 2, were chosen as representative macrocyclic hosts because both CB $n$

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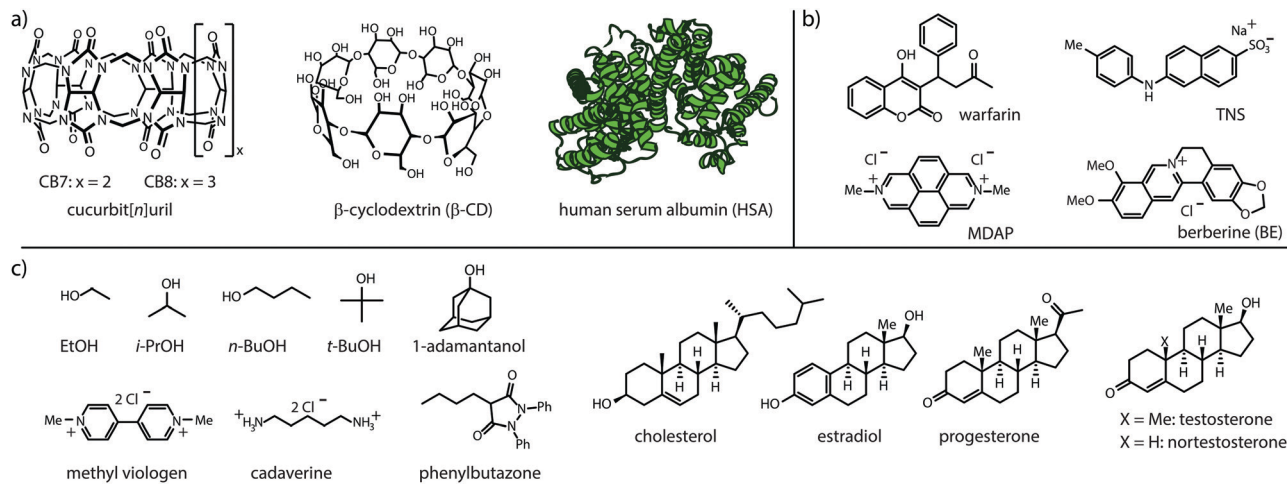
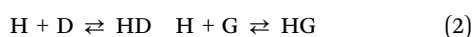


Fig. 2 Chemical structures of (a) hosts, (b) fluorescent indicator dyes, and (c) water-soluble and water-insoluble guests used in this study. See ESI† for solubilities of H, G and D, and photophysical properties of D and H>D.

and CDs are commercial, water-soluble and non-toxic,<sup>31–33</sup> and have received wide attention in the supramolecular, materials and pharmacological community.<sup>34</sup> Besides, they find use as solubility-enhancing excipients in pharmacology and industry,<sup>13,35–38</sup> making them ideal model hosts for the proposed GDA setup with insoluble guests. Human serum albumin (HSA) was selected as a model protein because of its important biological role as a carrier protein. Moreover, HSA is commercially available at a standardized high purity (fatty acid free grade) and provides a wide binding spectrum of hydrophobic drugs.<sup>39,40</sup> Twelve organic compounds ranging from hydrophilic, such as alcohols and cadaverine, to hydrophobic compounds such as steroids and phenylbutazone were selected as representative, non-chromophoric guests for CB $n$ , CD and HSA, see Fig. 2c.

In the herein introduced guest-displacement assay (GDA), a spectroscopically silent and potentially insoluble guest (G) is equilibrated with the host (H) to form a host-guest complex, H>G. Aliquots of an indicator (*e.g.* an emissive dye, D) are subsequently added, causing competitive displacement of G and formation of a host-dye complex, H>D, see Fig. 1b. At the first glance, GDA is simply a reversed IDA, however, the implications from this subtle change of the order of compound addition are important from both a fundamental and practical point of view.

The competitive binding network of H, G and D can be analyzed through the eqn (1)–(6), where  $K_a^{\text{HD}}$  and  $K_a^{\text{HG}}$  are the binding constants of the H>D and H>G complexes, respectively. H and G are assumed to be spectroscopically silent. Note that these mathematical equations also model the IDA titration experiments.<sup>15,16</sup>



$$K_a^{\text{HD}} = \frac{[\text{HD}]}{[\text{H}][\text{D}]} \quad K_a^{\text{HG}} = \frac{[\text{HG}]}{[\text{H}][\text{G}]} \quad (3)$$

$$[\text{H}]_0 = [\text{HD}] + [\text{H}] + [\text{HG}] \quad (4)$$

$$[\text{D}]_0 = [\text{HD}] + [\text{D}] \quad [\text{G}]_0 = [\text{HG}] + [\text{G}] \quad (5)$$

$$I_c = I^0 + I^{\text{HD}} \cdot [\text{HD}] + I^{\text{D}} \cdot [\text{D}] \quad (6)$$

From a photophysical point of view, it may appear counter-intuitive to raise the dye concentration in GDA instead of keeping it constant as in IDA. In fact, when GDA experiments are carefully designed to avoid inner filter effects, the increase in concentration of dye is no concern (Fig. 3a and best practice guide in the ESI†). Firstly, we verified that the GDA method is complementary to IDA when soluble components are used: for cucurbit[7]uril (CB7) and *n*-butanol as spectroscopically silent

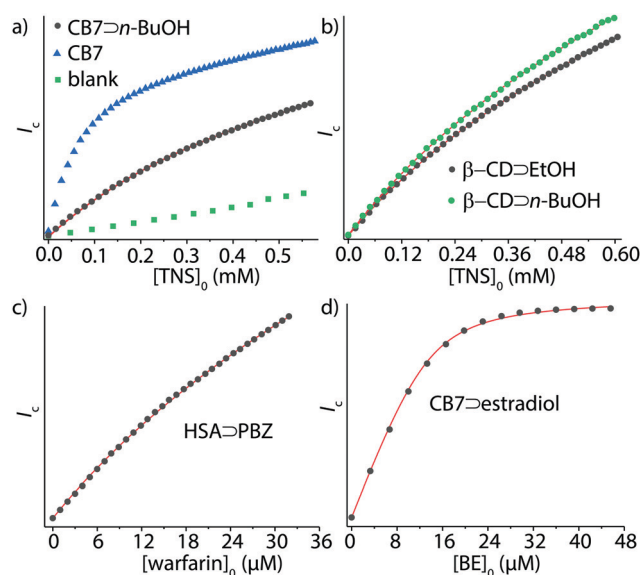


Fig. 3 Examples of GDA titration experiments: emission based titration curves of TNS (0–600  $\mu\text{M}$ ): (a) added to water (■), to a CB7 solution (51  $\mu\text{M}$ , ▲) and to a solution of CB7 (51  $\mu\text{M}$ ) and *n*-butanol (290  $\mu\text{M}$ ) (● and red fitting line); (b) added to a solution of  $\beta$ -CD (100  $\mu\text{M}$ ) and ethanol (7.05 mM, ●, red fitting line) and *n*-butanol (884  $\mu\text{M}$ , ●, red fitting line); (c) of warfarin (0–32  $\mu\text{M}$ ) added to a solution of HSA (10  $\mu\text{M}$ ) and PBZ (21  $\mu\text{M}$ ) (● and red fitting line) and (d) of BC (0–48  $\mu\text{M}$ ) added to a solution of CB7 (16.5  $\mu\text{M}$ ) and estradiol (13  $\mu\text{M}$ ) (● and red fitting line).



host and guest, and TNS as a fluorescent dye ( $\log K_a = 4.5$  for CB7 in water), GDA experiments in water yielded  $\log K_a = 4.9$ , which is in good agreement with  $\log K_a = 4.7$  by IDA, see Table 1 and Fig. 3a. Similarly, GDA and IDA yielded matching affinities for CB7 $\supset$ cadaverine complex formation,  $\log K_a = 8.4$  and 8.6. The performance of GDA was also tested for  $\beta$ -CD, CB8, and HSA as representative supramolecular hosts. For instance, GDA provided an accurate binding affinity for the moderately soluble anti-inflammatory drug phenyl butazone (PBZ,  $\log K_a = 5.8$ , Fig. 3c).

Importantly – unlike in DBA and IDA – with the GDA method insoluble guests can now be analyzed if soluble host–guest complexes can be formed. As a showcase example, we determined the  $K_a$  value of the nearly water-insoluble steroid estradiol (solubility  $S_{\text{H}_2\text{O}} \approx 9 \mu\text{M}$ , see Table S1, ESI $\dagger$ ) for CB7: firstly, the water-soluble CB7 $\supset$ estradiol complex was formed and the concentration of CB7 and estradiol was determined by  $^1\text{H-NMR}$ , see the ESI $\dagger$ . Aliquots of the CB7 $\supset$ estradiol stock solution were then titrated according to the GDA procedure with the water-soluble indicator dye berberine (BE) $^{41}$  (Fig. 3d).

From the binding isotherms,  $\log K_a = 6.3$  was obtained for the CB7 $\supset$ estradiol complex. Reassuringly, this binding strength is similar to that of the structurally-related, water-soluble estrane nortestosterone with CB7 ( $\log K_a = 7.0$ ) which was assessed by IDA and ITC experiments previously. $^{37}$  Likewise, the affinity of the more bulky, insoluble progesterone ( $S_{\text{H}_2\text{O}} \approx 33 \mu\text{M}$ ) for CB7 became available through the GDA titration method,  $\log K_a = 4.1$ . Alternatively, GDA titrations can be carried out at a low and constant vol% of cosolvent, as was demonstrated for the GDA of a CB7 $\supset$ cholesterol complex in a H $_2$ O/ethanol (99.5/0.5; v/v) mixture, see Table 1, while corresponding IDA titrations lead to a steady change of solvent:cosolvent ratio during the course of a titration.

During the GDA titration, the insoluble guest is displaced from the host cavity by the indicator dye that is subsequently stepwise added. Thus, one may wonder if the GDA method faces difficulties due to precipitation of the liberated insoluble guest. We have carefully tested for this scenario but have not observed any sign for precipitations. For instance, the homogeneous aqueous solutions of CB7 $\supset$ estradiol that was titrated with berberine dissolved in water, remained clear and did not scatter light even after the end point of the titration was reached because only micromolar quantities of the unbound guest were liberated. (Besides, precipitation or crystallization of organic compounds from saturated solutions can be slow.)

As a second major advantage of GDA is its superior performance for weakly binding guests, which was uncovered both by simulations and experiments. For instance, the affinity of *n*-butanol to  $\beta$ -CD could be determined by GDA (see Fig. 3b),  $\log K_a = 2.0$ , whereas IDA titrations required the addition of much larger amounts of the weakly binding guest and yielded poor mathematical fits, see Fig. S26 in the ESI $\dagger$ . (Besides, high concentrations of guests can cause deviations from unity activity coefficients and the experiments can face solubility limitations.)

Conversely, the IDA method should be chosen for soluble high-affinity guests. To exemplify, GDA yielded only an approximate binding constant for the  $\beta$ -CD $\supset$ AdOH complex because

Table 1 Experimental binding affinities determined by GDA and IDA in aqueous media

Guest <sup>a</sup>	Host <sup>a</sup>	Dye <sup>a</sup>	$\log K_a^b$	Method <sup>c</sup>
Ethanol	CB7	TNS	2.49 $\pm$ 0.01	GDA
<i>n</i> -Butanol	CB7	TNS	4.89 $\pm$ 0.04	GDA
			4.69 $\pm$ 0.02	IDA
Cadaverine	CB7	BE	$\sim 8^d$	GDA
			8.37 $\pm$ 0.05	IDA
		MDAP	8.64 $\pm$ 0.03	GDA
Progesterone	CB7	TNS	4.77 $\pm$ 0.08	GDA
Estradiol	CB7	BE	6.25 $\pm$ 0.11	GDA
Cholesterol	CB7	BE	5.91 $\pm$ 0.04	GDA
Methyl viologen	CB7	BE	8.84 $\pm$ 0.04	IDA
		MDAP	8.78 $\pm$ 0.01	GDA
MDAP	CB7	BE	9.43 $\pm$ 0.02	IDA
Nortestosterone	CB8	BE <sup>e</sup>	8.19 $\pm$ 0.09	GDA
Ethanol	$\beta$ -CD	TNS	1.93 $\pm$ 0.01	GDA
<i>i</i> -Propanol	$\beta$ -CD	TNS	2.27 $\pm$ 0.02	GDA
<i>n</i> -Butanol	$\beta$ -CD	TNS	2.00 $\pm$ 0.04	GDA
			$\sim 2^d$	IDA
<i>t</i> -Butanol	$\beta$ -CD	TNS	2.26 $\pm$ 0.08	GDA
1-Adamantanol	$\beta$ -CD	TNS	5.01 $\pm$ 0.08	IDA
			$\sim 5^d$	GDA
Phenylbutazone	HSA	Warfarin	5.83 $\pm$ 0.04 <sup>f</sup>	GDA

<sup>a</sup> See Fig. 1 for chemical structures of guest, hosts and dyes. See Table S3 in the ESI for H $\supset$ D affinities. Values are available at “suprabank.org”.

<sup>b</sup> Binding affinities,  $K_a$  in  $\text{M}^{-1}$ , in deionized water; errors (StDev) were obtained from triplicate experiments, see Table S3 (ESI) for details. A mixture of H $_2$ O/ethanol (99.5/0.5; v/v) was used for CB7 $\supset$ cholesterol. Phosphate buffer saline (PBS) was used for HSA. <sup>c</sup> DBA carried out by titrating the dye with the host, or the host with the dye. <sup>d</sup> Values of  $\log K_a^{\text{HG}}$  and  $\log K_a^{\text{HD}}$  are outside the recommended range for GDA or IDA, see text. <sup>e</sup> CB8 $\supset$ BE $_2$   $\log K_a = 13.01$  ( $\text{M}^{-1}$ ).<sup>42</sup> <sup>f</sup>  $\log K_a = 5.41$ , via Scatchard plot.<sup>40</sup>

the affinity of the commercially available indicator dye TNS $^{41}$  (3.4), lay outside the recommended range for GDA, see below as well as Fig. 4. IDA titration gave reliably  $\log K_a = 5.0$  for this host–guest pair. Use of recently developed high-affinity indicator dyes will increase the scope of the GDA method for cyclodextrins. $^{43}$  Despite the complementarity of GDA and IDA for  $K_a$  value determination, GDA and IDA do not behave as exact “mirror images”, *i.e.* there are different requirements for the selection of suitable indicator dyes. Explicit mathematical simulations that are described in the ESI $\dagger$  showed that GDA is best suited for  $\log K_a^{\text{HG}} + 2 \geq \log K_a^{\text{HD}} \geq \log K_a^{\text{HG}} - 1$  (see Fig. 4 and Fig. S1–S7 in the ESI $\dagger$ ). On one hand, the indicator dye should not bind by more than a factor of 10 in  $K_a$  weaker than the guest, otherwise the indicator cannot efficiently displace the guest from the host–guest complex, resulting in a flat binding isotherm that is not accurately fitable. On the other hand, the indicator dye can bind up to a factor of 100 stronger than the guest and still produce a fitable binding isotherm. For instance, if a guest with a suspected binding affinity of  $10^6 \text{ M}^{-1}$  is tested by the GDA method, an indicator dye with a  $K_a$ -range of  $10^5$  to  $10^8 \text{ M}^{-1}$  should be selected (Fig. 4). In contrast, in IDA fitable binding isotherms are obtained if  $\log K_a^{\text{HG}} + 1 \geq \log K_a^{\text{HD}} \geq \log K_a^{\text{HG}} - 2$  holds true.

Thus, for the same guest with  $K_a \sim 10^6 \text{ M}^{-1}$  a suitable IDA indicator dye can be found in the affinity range of  $10^4$  to  $10^7 \text{ M}^{-1}$ . Experimentally, these predictions were verified for the example case of CB7 as the host and cadaverine as the guest, that required the use of MDAP $^{44}$  and berberine as indicator dyes for





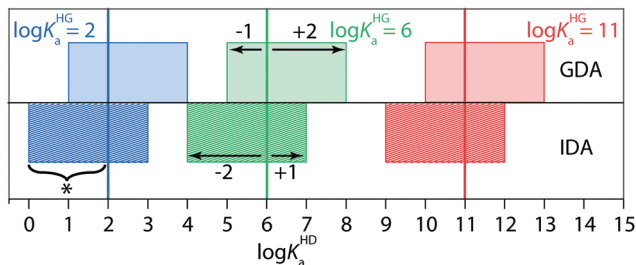


Fig. 4 Performance analysis of the competitive binding assays GDA and IDA (see best practice guide in the ESI†).

GDA and IDA binding titrations, respectively (Table 1 and Fig. S18 (ESI†), for chemical structures of the dyes see Fig. 2b).

In conclusion, it was shown that the guest-displacement assay enables the binding affinity determination of spectroscopically silent host-guest and protein-ligand pairs. The GDA method is applicable also for insoluble, e.g. hydrophobic, guests and for weakly binding guests, both of which escaped binding affinity determinations by state-of-the-art direct-binding and indicator-displacement assays. Moreover, the GDA method will be beneficial for conducting full binding titrations with gaseous hydrocarbons<sup>45</sup> and noble gases<sup>7,46</sup> as guests whose concentration can be readily fixed to their solubility. The extension to other hosts and supramolecular systems that bind guest molecules, e.g. cages, will be possible.

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## Conflicts of interest

There are no conflicts of interest to declare.

## Notes and references

- H. Adams, F. J. Carver, C. A. Hunter, J. C. Morales and E. M. Seward, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1542–1544.
- I. K. Mati and S. L. Cockcroft, *Chem. Soc. Rev.*, 2010, **39**, 4195–4205.
- P. Thordarson, *Chem. Soc. Rev.*, 2011, **40**, 1305–1323.
- E. Persch, O. Dumele and F. Diederich, *Angew. Chem., Int. Ed.*, 2015, **54**, 3290–3327.
- F. Biedermann and H.-J. Schneider, *Chem. Rev.*, 2016, **116**, 5216–5300.
- J. Řezáč and P. Hobza, *Chem. Rev.*, 2016, **116**, 5038–5071.
- S. He, F. Biedermann, N. Vankova, L. Zhechkov, T. Heine, R. E. Hoffman, A. De Simone, T. T. Duignan and W. M. Nau, *Nat. Chem.*, 2018, **10**, 1252–1257.
- F. Jia, H. Hupatz, L. P. Yang, H. V. Schroder, D. H. Li, S. Xin, D. Lentz, F. Witte, X. Xie, B. Paulus, C. A. Schalley and W. Jiang, *J. Am. Chem. Soc.*, 2019, **141**, 4468–4473.
- D.-S. Guo, V. D. Uzunova, K. I. Assaf, A. I. Lazar, Y. Liu and W. M. Nau, *Supramol. Chem.*, 2016, **28**, 384–395.
- K. I. Assaf and W. M. Nau, *Angew. Chem., Int. Ed.*, 2018, **57**, 13968–13981.

- P. Remón, D. González, M. A. Romero, N. Basilio and U. Pischel, *Chem. Commun.*, 2020, **56**, 3737–3740.
- F. Hof, S. L. Craig, C. Nuckolls and J. J. Rebek, *Angew. Chem., Int. Ed.*, 2002, **41**, 1488–1508.
- J. Murray, K. Kim, T. Ogoshi, W. Yao and B. C. Gibb, *Chem. Soc. Rev.*, 2017, **46**, 2479–2496.
- E. C. Hulme and M. A. Trevethick, *Br. J. Pharmacol.*, 2010, **161**, 1219–1237.
- L. You, D. Zha and E. V. Anslyn, *Chem. Rev.*, 2015, **115**, 7840–7892.
- S. Sinn and F. Biedermann, *Isr. J. Chem.*, 2018, **58**, 357–412.
- S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne and E. V. Anslyn, *Acc. Chem. Res.*, 2001, **34**, 963–972.
- D. J. Cram and G. M. Lein, *J. Am. Chem. Soc.*, 1985, **107**, 3657–3668.
- S. Zhang, L. Grimm, Z. Miskolczy, L. Biczók, F. Biedermann and W. M. Nau, *Chem. Commun.*, 2019, **55**, 14131–14134.
- S. Dixit, J. Crain, W. C. K. Poon, J. L. Finney and A. K. Soper, *Nature*, 2002, **416**, 829–832.
- M. Rosés, C. Ràfols, J. Ortega and E. Bosch, *J. Chem. Soc., Perkin Trans. 2*, 1995, 1607–1615.
- V. Francisco, A. Piñeiro, W. M. Nau and L. García-Río, *Chem. – Eur. J.*, 2013, **19**, 17809–17820.
- F. Sommer, Y. Marcus and S. Kubik, *ACS Omega*, 2017, **2**, 3669–3680.
- S. Liu, P. Y. Zavalij and L. Isaacs, *J. Am. Chem. Soc.*, 2005, **127**, 16798–16799.
- D. Shetty, J. K. Khedkar, K. M. Park and K. Kim, *Chem. Soc. Rev.*, 2015, **44**, 8747–8761.
- J. Kim, I. S. Jung, S. Y. Kim, E. Lee, J. K. Kang, S. Sakamoto, K. Yamaguchi and K. Kim, *J. Am. Chem. Soc.*, 2000, **122**, 540–541.
- W. M. Nau, M. Florea and K. I. Assaf, *Isr. J. Chem.*, 2011, **51**, 559–577.
- S. J. Barrow, S. Kaseira, M. J. Rowland, J. del Barrio and O. A. Scherman, *Chem. Rev.*, 2015, **115**, 12320–12406.
- K. A. Connors, *J. Pharm. Sci.*, 1995, **84**, 843–848.
- J. Szejtli, *Chem. Rev.*, 1998, **98**, 1743–1754.
- V. D. Uzunova, C. Cullinane, K. Brix, W. M. Nau and A. I. Day, *Org. Biomol. Chem.*, 2010, **8**, 2037–2042.
- K. M. Park, J. A. Yang, H. Jung, J. Yeom, J. S. Park, K. H. Park, A. S. Hoffman, S. K. Hahn and K. Kim, *ACS Nano*, 2012, **6**, 2960–2968.
- M. J. Rowland, C. C. Parkins, J. H. McAbee, A. K. Kolb, R. Hein, X. J. Loh, C. Watts and O. A. Scherman, *Biomaterials*, 2018, **179**, 199–208.
- M. E. Davis and M. E. Brewster, *Nat. Rev. Drug Discovery*, 2004, **3**, 1023.
- M. E. Brewster and T. Loftsson, *Adv. Drug Delivery Rev.*, 2007, **59**, 645–666.
- D. Ma, G. Hettiarachchi, D. Nguyen, B. Zhang, J. B. Wittenberg, P. Y. Zavalij, V. Briken and L. Isaacs, *Nat. Chem.*, 2012, **4**, 503–510.
- A. I. Lazar, F. Biedermann, K. R. Mustafina, K. I. Assaf, A. Hennig and W. M. Nau, *J. Am. Chem. Soc.*, 2016, **138**, 13022–13029.
- N. Basilio and U. Pischel, *Chem. – Eur. J.*, 2016, **22**, 15208–15211.
- Å. Frostell-Karlsson, A. Remaeus, H. Roos, K. Andersson, P. Borg, M. Hämäläinen and R. Karlsson, *J. Med. Chem.*, 2000, **43**, 1986–1992.
- V. Maes, Y. Engelborghs, J. Hoebeke, Y. Maras and A. Vercruyssen, *Mol. Pharmacol.*, 1982, **21**, 100–107.
- R. N. Dsouza, U. Pischel and W. M. Nau, *Chem. Rev.*, 2011, **111**, 7941–7980.
- S. Sinn, E. Spuling, S. Bräse and F. Biedermann, *Chem. Sci.*, 2019, **10**, 6584–6593.
- K. I. Assaf, O. Suckova, N. Al Danaf, V. von Glasenapp, D. Gabel and W. M. Nau, *Org. Lett.*, 2016, **18**, 932–935.
- V. Sindelar, M. A. Cejas, F. M. Raymo and A. E. Kaifer, *New J. Chem.*, 2005, **29**, 280–282.
- M. Florea and W. M. Nau, *Angew. Chem., Int. Ed.*, 2011, **50**, 9338–9342.
- G. Huber, T. Brotin, L. Dubois, H. Desvaux, J.-P. Dutasta and P. Berthault, *J. Am. Chem. Soc.*, 2006, **128**, 6239–6246.

