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General Principles and Strategies for Salting-Out Informed by the Hofmeister Series

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Supporting Information

ABSTRACT: Overarching principles for salting-out extraction are long-established but poorly disseminated. We highlight the opportunity for more widespread application of this technique using the Hofmeister series as a foundational basis for choosing the right salt. The power of this approach is exemplified by the aqueous workup of a highly water-soluble nucleoside in which the use of sodium sulfate allowed for high recoveries without relying on back extraction.

INTRODUCTION

While developing a synthesis for the HCV drug uprifosbuvir,[1] a number of nucleoside intermediates with high water solubilities were encountered, including chlorouridine **1**[1b] (Figure 1).

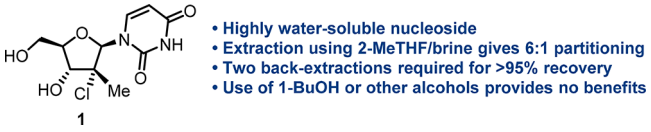

Figure 1. Challenging aqueous workup of nucleoside **1**.

Consequently, its purification by aqueous workup proved challenging due to poor partitioning. Salting-out with sodium chloride only gave a 6:1 distribution between the organic and aqueous phases, and two back extractions were still necessary to obtain >95% recovery. Although effective in the lab for early development, this series of operations would be labor-intensive and wasteful at manufacturing scale. Hence, we aimed to redesign the process while setting a high threshold for success: a single extraction with >30:1 partitioning. Literature precedents on the extraction of similar nucleosides did not provide any clues as how to accomplish this.[2] Our stance was that developing a more efficient extraction[3] would require a judicious choice of conditions based on a deep understanding of the underlying physiochemical properties. More specifically, we proposed that the salt/solvent combination was suboptimal and should be re-examined to maximize the salting-out effect. A cursory inspection of the organic and process chemistry primary and reference literature revealed a surprising absence of detailed information regarding the topic of salting-out extraction. However, a more thorough search through older literature and in journals considered out of field to organic chemists actually revealed a wealth of useful information on salting-out that is currently underappreciated. Moreover, there is a lack of pedagogical source material that addresses difficult aqueous workups in general, which was called out in a recent paper by Hill and Sweeney.[4] In it, they offer sage advice and advocate for using a rational problem-solving approach, but salting-out is only briefly discussed. In view of this incongruity, the principal aims of this paper are to educate the organic chemistry community on the applied and theoretical aspects of salting-out extraction, provide practical laboratory guidance, and promote its use through a brief review followed by an illustrative case study with nucleoside **1**.

Enhanced Extraction Techniques for Water-Soluble Compounds. The presence of multiple polar functionalities (e.g., amides, alcohols, amines, etc.) generally increases the water solubility of organic compounds, often leading to difficulties in aqueous workups. To address these situations, a number of techniques that fall under the umbrella of "enhanced extraction"[4] have been developed (Table 1): (1) Optimization of the extraction solvent and cosolvent (e.g., 1-BuOH or other water-immiscible alcohols)[5] can improve partitioning but usually only to a modest extent, if at all.[6] (2) Continuous liquid−liquid extraction overcomes poor partitioning by continual renewal of fresh solvent.[7] Although continuous processing can offer many advantages, it requires significant capital investment and limits the portability of the process. (3) When ionizable or carbonyl functional groups are present, reactive extraction[8]—executed by addition of lipophilic acids[8b]/bases[8c] or nucleophiles,[8d] respectively—can be a very powerful method to achieve selective phase distributions, provided that there are no interfering functional groups present. (4) Selective extractions have been developed based on a variety of noncovalent interactions between the solute and an additive: hydrogen bonding of the solute with phosphine oxides or phosphonates,[9a] chiral recognition,[9b,c] or templated anion binding of nucleotides.[9d] (5) Salting-out extraction[10] can greatly facilitate the recovery of organic compounds with minimal changes to an existing batch process and is operationally simple.[11] An added benefit is that salts will usually increase the density of the aqueous layer[12] making emulsions resulting from equi-dense layers, in cases of nonhalogenated solvents, unlikely. (6) Finally, aqueous biphasic or two-phase extraction, a subcategory of salting-out, is used for the isolation of extremely water-soluble components. This

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Table 1. Enhanced Liquid–Liquid Extraction Techniques for Highly Water-Soluble Compounds

extraction method	additives required	strengths	drawbacks
protic organic	1-BuOH, 2-BuOH, or other alcohol	simple to implement	composition of organic layer changes with each wash limited optimization possible
continuous	none	high recovery small footprint	specialized equipment
reactive	lipophilic base/acid or other reactive partner	highly efficient highly selective	functional group specific
affinity	complexing host molecule	highly selective	host molecules highly engineered and must be subsequently removed
salting-out	salt	highly efficient simple to implement increases density of the aq. layer	residual salt in organic layer
aqueous biphasic	polymer, ionic liquid, etc. + salt	useful for extremely water-soluble components	additive must be separated

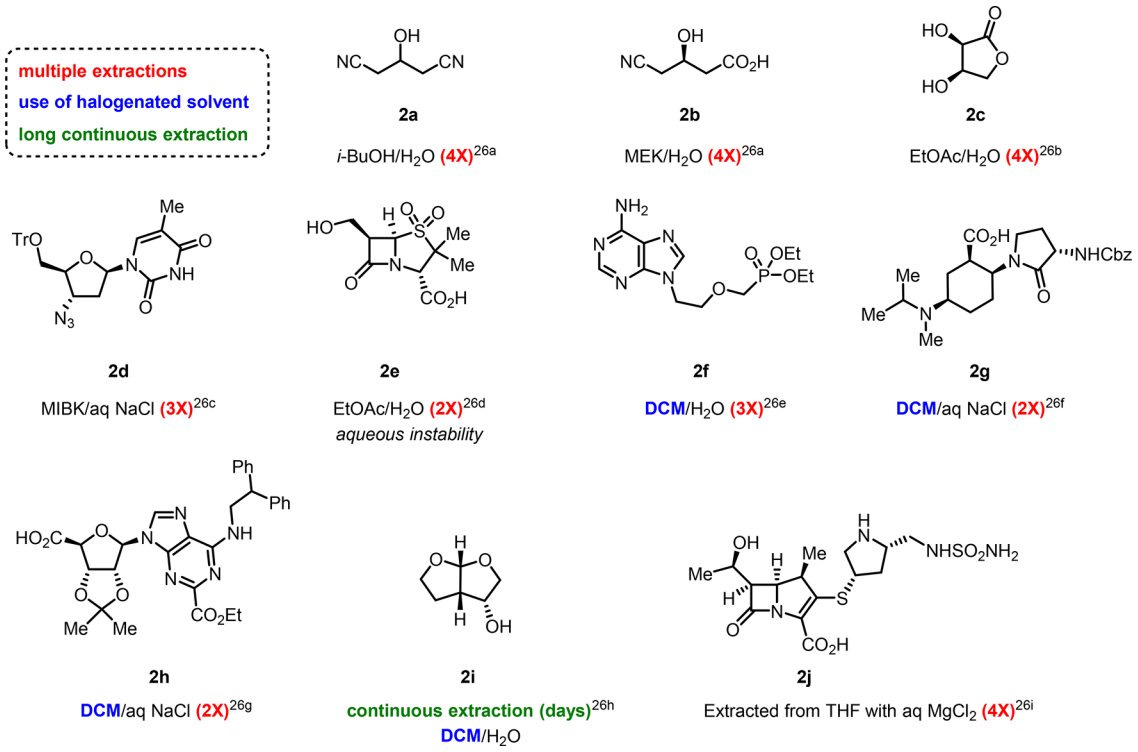

Figure 2. Examples from OPR&D of difficult extractions with water-soluble compounds.

approach takes advantage of a polymer (typically PEG),[13] low molecular weight alcohol, deep-eutectic solvent combination,[14] or ionic liquid[15] that, in combination with a salt and water, results in two immiscible phases. The high polarity of these solvent mixtures allows for the purification of small polar organics (e.g., 1,3-propanediol, 2,3-butanediol, lactic acid) from biomass or fermentation broth,[16] biomolecules (proteins, DNA, RNA) and even large biologic particles (cells, organelles, bacteria, viruses).[17] While each of the above technologies has strengths and potential liabilities, salting-out extraction is simple to implement and can provide great benefits with little additional cost. Furthermore, because of its utility, salting-out has found widespread applications including protein isolation by precipitation with ammonium sulfate,[18] protein crystallization,[19] industrial manufacturing processes for dyes,[20] soaps,[21] and caprolactam,[22] textile dyeing,[23] as well as in analytical chemistry for extracting analytes from biological materials.[24,25]

Potential Benefits of Salting-out in Pharmaceutical Process Chemistry. To illustrate the need for improved extraction methods, examples of pharmaceuticals or intermediates with high water solubility that posed difficulties in liquid–liquid extraction are presented (Figure 2).[26] In many of these processes, multiple back-extractions were required to compensate for poor partitioning (**2a–2h, 2j**). At best, this approach is inefficient, but it can also present a significant

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liability when an intermediate with limited water stability such as sulbactam **2e** is subjected to prolonged aqueous exposure. In some cases, multiple extractions were necessary even while salting out with brine (**2d, 2g, 2h**). In a biocatalytic process, alcohol **2i** was extracted into DCM from water using continuous counter-current exchange over multiple days. In a unique case where salting-in was utilized, an aqueous solution of doripenem (**2j**) and $MgCl_2$ was washed with THF to remove lipophilic impurities. Although ultimately effective, four aqueous extractions of the organic layer were required due to incomplete partitioning.

In the preceding examples, most operations were performed on multikilogram scale, suggesting a substantial investment in process optimization. However, suboptimal end points were likely reached from the perspective of efficiency (high solvent usage, significant aqueous losses, long time cycles)[27] and green solvent selection (use of DCM).[28] In 2011, the ACS GCI Pharmaceutical Roundtable identified separation technologies, solvent choice, and process intensification as three of the top five needs for green engineering and manufacturing.[29] This emphasis is justified by the fact that separations typically contribute to 40−90% of the process mass intensity (PMI)[30] of a process. As indicated, salting-out extraction will be our focus, and we aver that the technique has great untapped potential. While avoiding extraction altogether will always be ideal,[31] it is our assertion that when necessary, skillful application of salting-out will enable the development of processes with greener attributes.[32] Moreover, a greater appreciation of these concepts is essential for organic chemists, especially those engaged in process research, who strive to minimize aqueous losses during workups and are in pursuit of process intensification.[33]

Definitions and Historical Context. Salting-out is formally defined as the phenomenon when the solubility of a nonelectrolyte substance in water *decreases* with *increasing* salt concentration.[34,35] Conversely, salting-in is defined for instances when the solubility of a nonelectrolyte in water *increases* with *increasing* salt concentration.[36] The relative effectiveness of salting-out or -in is traditionally quantified by use of the Setschenow equation (eq 1) in which S_0 is the solubility of the solute in pure water, S is the solubility of the solute in the salt solution, K_s is the salting out or Setschenow constant, and C is the concentration of the salt.[37] K_s will be positive when salting-out occurs and negative when salting-in occurs.

$$\log \frac{S_0}{S} = K_s C \tag{1}$$

The concentration distribution of a solute (A) between two immiscible liquid phases (e.g., organic and aqueous) is described by the unitless distribution ratio D (eq 2).[38,39]

$$D = \frac{[A]_{org}}{[A]_{aq}} \tag{2}$$

Since much of the literature on salting-out is based solely on aqueous solubilities, it is necessary to understand the thermodynamic relationship between solubility and biphasic partitioning. For establishing thermodynamic relationships, the concept of solution activity must be invoked. The activity of a solute in solution at a given temperature is defined as the product of its concentration and an activity coefficient (γ) (eq 3). For a biphasic mixture at equilibrium, the solute's activities will be equal in the two phases (eq 4).

$$a = \gamma[A] \tag{3}$$

$$a_{org} = a_{aq} \text{ (at equilibrium)} \tag{4}$$

Combination of eqs 3 and 4 provides eq 5 and by rearranging the terms we arrive at eq 6. Thus, partitioning is directly related to the ratio of activity coefficients in ideal aqueous and organic solutions (i.e., at the limit of infinite dilution).[40] It follows that the distribution will be independent of the total concentration of dissolved solute unless there is significant self-association or ionization.[41]

$$\gamma_{org}[A]_{org} = \gamma_{aq}[A]_{aq} \text{ (at equilibrium)} \tag{5}$$

$$\frac{[A]_{org}}{[A]_{aq}} = \frac{\gamma_{aq}}{\gamma_{org}} = D \tag{6}$$

The value of D is often dictated by the aqueous solubility of the solute.[42] However, the thermodynamic relationships become complex when the organic solvent is partially miscible with water or there is significant self-association of the solute.[43] Despite these potential complications, as a first approximation, the lessons learned from salting-out in water can be applied to biphasic partitioning.[44]

Practical guidance on salting-out extraction in the context of organic synthesis was first described in some detail within Ludwig Gattermann's influential 1894 monograph entitled "Die Praxis des Organischen Chemikers", which was translated into English by Schober and Babasinian as "Practical Organic Chemistry Methods" in 1898.[45] An entire section is devoted to salting-out, which begins: "A very valuable method to induce substances dissolved in water to separate out is known as "salting out"." Gattermann advocates the general use of NaCl, KCl, K_2CO_3, $CaCl_2$, NH_4Cl, Na_2SO_4, or NaOAc for this purpose. More specifically, K_2CO_3 is identified as being most proficient at separating acetone or alcohols from water,[46] while NaCl is recommended when extracting solutes from aqueous solutions with diethyl ether. At the end of the section, he states: "Unfortunately the method of "salting out" has not been so generally adopted in scientific laboratories as it deserves, while in the laboratory of technical chemists it has long been in daily use." Curiously, and perhaps foreshadowed by Gattermann's remark, subsequent generations of practical organic chemistry textbooks have greatly abbreviated its treatment, and as a consequence, downplayed its usefulness. As such, present-day authoritative references on extraction offer little advice for dealing with water-soluble compounds beyond trying NaCl or another salt—typically without any practical guidance, theory, or general references.[11] Of particular importance, there is far more nuance and depth to salting-out than has been conveyed in these sources or by Gattermann.

Specific Ion Effects and the Hofmeister Series. The degree of salting-out, reflecting the activity coefficient of the aqueous solution, is sometimes attributed to aqueous ionic strength, as Debye−Hückel theory provides a direct mathematical link.[47] However, without the inclusion of additional empirical parameters, this relationship only holds true up to 0.1 M in salt concentration. This upper bound greatly limits its relevance, since salting out is typically performed with much higher salt concentrations.[37b,48] At these higher concentrations, *specific ion effects*[49] are observed instead, which is the general and consistent ordering of anions in relation to their salting-out strengths.[35] For example, in one of the earliest studies on

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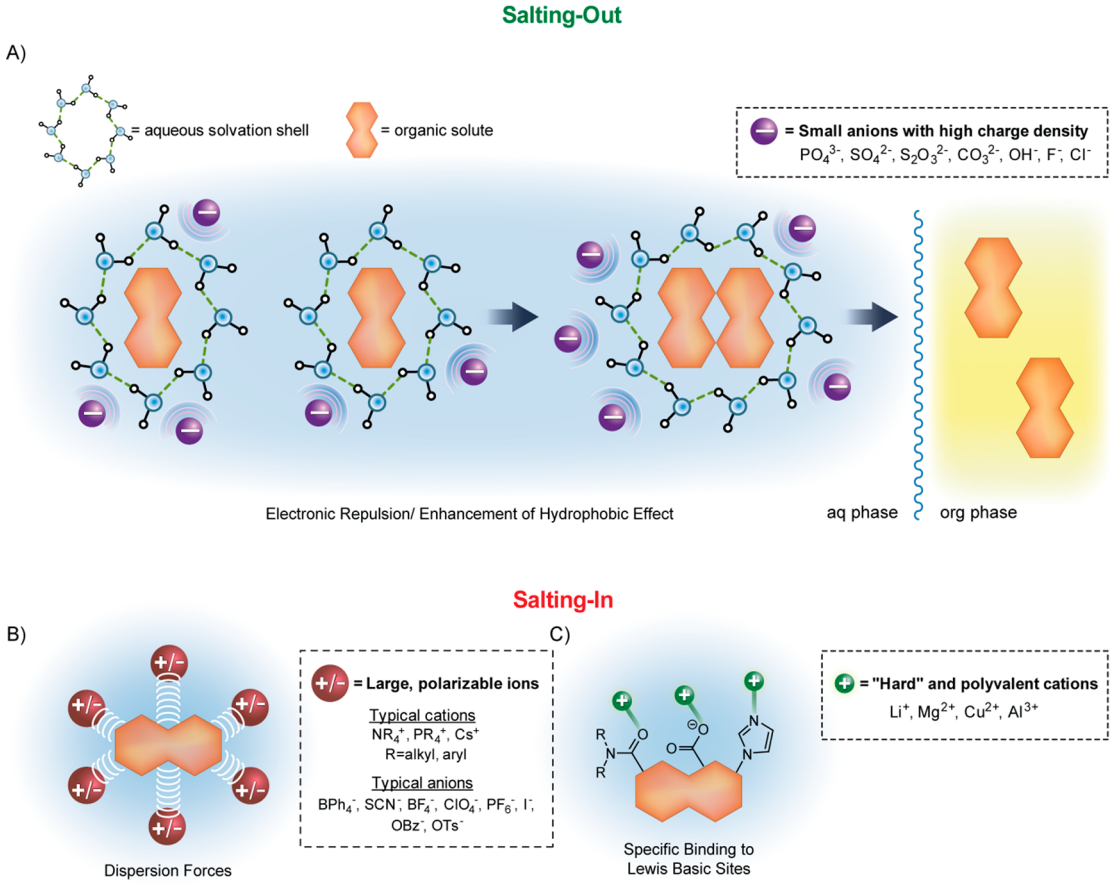

Figure 3. Molecular forces that dictate the aqueous solubility of organic solutes.

salting-out, the following sequences were found for decreasing the water solubility of phenylthiourea with respect to the anions: $OH^- \approx SO_4^{2-} \approx CO_3^{2-} > ClO_3^- \approx BrO_3^- \approx Cl^- \approx OAc^- \approx IO_3^- > Br^- \approx I^- > NO_3^-$; and the cations: $Na^+ > K^+ > Li^+ \approx Ba^{2+} \approx Rb^+ \approx Ca^{2+} \approx Ni^{2+} \approx Co^{2+} \approx Mg^{2+} \approx Fe^{2+} \approx Zn^{2+} \approx Cs^+ \approx Mn^{2+} \approx Al^{3+} > NH_4^+ > H^+$.[34a] Conspicuously, it has been observed in the vast majority of cases that the anion has a much larger effect than the cation and the ordering of anions in terms of salting power is nearly constant.[35,50] Anions in the beginning of this series through approximately Cl^- will salt-out and are often called kosmotropes (order-making), while anions near the end of this series will salt-in and are often called chaotropes (chaos-making). The sequence for cations, however, is more variable and sensitive to the nature of the solute, particularly when polar functional groups are present.[35a,51] A limited number of studies have explored the scope of salting-out/in with respect to the solute's structure, but some general trends have been established. The magnitude of specific ion effects will generally increase with the following attributes of the nonelectrolyte: (1) higher polarizability,[52] (e.g., extended aromatics), (2) larger molecular size/volume,[34b,35a,53] and (3) lower polarity.[54]

Salting effects have further significance because they trend closely with the Hofmeister series.[55,56] This phenomenon is the empirical ordering of salts based on the minimum concentration needed to cause protein precipitation from an aqueous solution. The sequence established for anions ordered from most to least precipitating is $CO_3^{2-} > SO_4^{2-} > S_2O_3^{2-} > H_2PO_4^- > F^- > Cl^- > Br^- \approx NO_3^- > I^- > ClO_4^- > SCN^-$; and for cations: $(CH_3)_4N^+ > Cs^+ > Rb^+ > NH_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+}$. Strikingly, the sequence for anions parallels the salting-out series for small molecules while the sequence for cations is rearranged (vida infra). The Hofmeister series also has far-reaching importance[57] with relevance to diverse fields including aquatic[35b,58] and atmospheric chemistry,[59] microbiology,[60] physiology and medicine,[61] biochemistry,[62] food chemistry,[63] anion binding and host–guest interactions,[64] chromatography,[65] and polymer behavior.[66] Although the observations by Hofmeister may appear unrelated to the current discussion, many of the same underlying chemical forces are responsible for the Hofmeister series and salting-out/in of small molecules. The importance of these effects on solution chemistry is likely why the Hofmeister series is so prevalent throughout the physical and biological sciences.

Mechanisms for Salting Out/In. Many theories have been proposed over the years to account for salting-out, and the exact mechanism is still debated.[61,67] However, in a simplistic model, dissolved anions of high charge density cause salting-out through a combination of electronic repulsion[35a,68] and enhancement of the hydrophobic effect[69,70] (Figure 3a). The hydrophobic effect in pure water causes solute aggregation to minimize the entropic penalty associated with highly ordered structure at the solute–water interface.[71] Presumably, in the presence of salts of high charge density, the analogous surface contacts are more ordered and incur an even larger entropic penalty.[72] Therefore, fully water-solvated states are disfavored, causing them to aggregate[72b,c,e] and then exit the aqueous phase. For solutes that are highly polar and water-soluble, the relative contributions of electronic repulsion and hydrophobic

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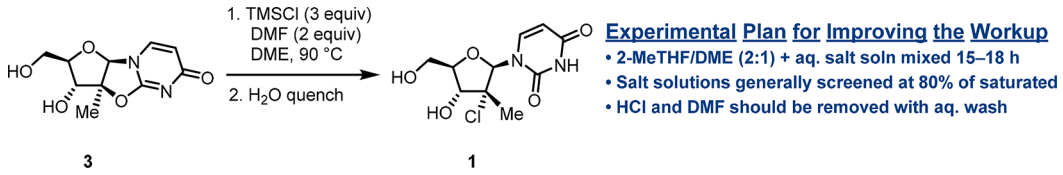
Scheme 1. Reaction Conditions for the Ring-Opening Hydrochlorination of Anhydrouridine 3



3 1

effects are poorly understood and may be different than those in the more-studied nonpolar model systems.

For salting-in, there are two specific interactions responsible depending on the charge density of the ion. Lipophilic ions, or more generally ions with low charge density, have been shown to bind solutes through nonlocalized attractive dispersion forces (Figure 3b). These ions behave like surfactants by adding charge to the surface of the solute,[69d,73] resulting in greater water solubility, but without formation of micelles.[74] Examples include cesium, tetraalkylammonium,[36] pyridinium, guanidinium, and tetraphenylphosphonium cations as well as haloacetate,[75] benzoate,[74] tosylate, pentachlorophenolate, tetraphenylborate, thiocyanate, perchlorate, and iodide anions.[73a] Salts of these ions are typically extremely water-soluble (>100 mg/mL), and they all salt-in strongly. The subset of these with both polar and nonpolar regions are called hydrotropes and have proven useful in a number of applications that require increased water solubility of solutes including chemical separations,[76a] drug formulation,[76b] wood pulp processing,[76c] and running reactions in water.[76c]

Ions with high charge density and engaged in localized binding to polarized or charged functional groups[77] are depicted in Figure 3c. Specific binding is likely the reason that the relative position of cations in a salting sequence depends on the functional groups present in the solute or protein.[51a] The well-known technique for removal of DMF or other polar aprotic solvents from an organic solution with aqueous LiCl takes advantage of this effect.[78] Likewise, when pyridines are washed away from organic solutions using aqueous $CuSO_4$, similar tight specific binding phenomena are responsible.[79] Li^+, Mg^{2+}, Cu^{2+}, and Al^{3+} are the cations that most often exhibit this behavior but the magnitude of salting-in will usually not be as great as for lipophilic ions. This difference in salting strength can be attributed to binding stoichiometry, as hard cations usually bind in a 1:1 ratio with a Lewis basic functional group, while multiple lipophilic ions can simultaneously engage a solute.

Independent of the interactions involved, a general guideline is that small, multiply charged anions with a high charge density salt-out strongly. Small, hard cations may or may not have much of an effect depending on the specific functional groups present in the solute. Anions or cations that are large or have diffuse charge density will always salt-in. Combinations of different types of anions and cations will have an intermediate effect but if one ion is highly lipophilic, it will likely have the dominant effect.

Mathematical Treatment of Partitioning Data. While the concentration-based partition measurement (D) is perfectly suitable for representing distributions in many situations, it fails to account for varying volume ratios of the phases. When the system contains a partially water-miscible solvent, phase ratios can vary dramatically. To express the total mass partitioning, D can be multiplied by the ratio of volumes to obtain the mass distribution ratio, noted as D_m (eq 7).[38]

$$D_m = D \times \frac{V_{org}}{V_{aq}} \tag{7}$$

A Setschenow equation for two-phase systems can also be used to express salting-out power (eq 8), where D_0 is the distribution between organic solvent and pure water, and D is the partitioning between organic solvent and salt water, K_{sd} is the Setschenow distribution constant, and C is the salt concentration.[35a]

$$\log \frac{D}{D_0} = K_{sd}C \tag{8}$$

In cases when solute self-association becomes significant, a modified version of eq 8 is required.[35a,80a,b] Even then, eq 8 does not always adequately fit the data, and in these cases, more sophisticated treatments are necessary.[80c,d] Given these factors, coupled with the large number of data points required to establish salting constants, and our need to screen broadly, we opted to treat our data differently. Instead, we took the natural logarithm of D_m to linearize the data and then normalized the resulting values by dividing by salt concentration (C, g/mL) to arrive at eq 9 in units of $(g/mL)^{-1}$. With the aim toward an efficient process with low cost and PMI, D_{norm} provides a sense of the efficiency in terms of the quantity of salt required to reach a given distribution.

$$D_{norm} = \frac{\ln(D_m)}{C} \tag{9}$$

RESULTS AND DISCUSSION

Salt Effects on Partitioning of 1. Chlorouridine **1** was prepared by reacting anhydrouridine **3**[1b] with an excess of TMSCl in DME/DMF at 90 °C (Scheme 1). The resulting product mixture was partially concentrated followed by an aqueous quench/workup, in which the mixture was diluted with 2-MeTHF and washed with brine. The purpose of the workup, in addition to silyl deprotection, was to remove HCl and DMF as they interfered with the subsequent crystallization. A cursory examination of alternate solvent mixtures for the extraction of chlorouridine **1** from 20 wt % aq. NaCl did not produce any promising leads for improving partitioning beyond 6:1 (org/aq). Therefore, the solvent was held constant (2:1 2-MeTHF/DME, v/v) for the investigation of salt effects. When choosing salts to examine, our sole criterion was good solubility in water (>10 wt %) at 20−25 °C. While a manufacturing process would ultimately require a nontoxic and inexpensive salt, we chose to look more broadly to gain fundamental insights. The concentrations of the salt solutions prepared were 80% of saturation, unless this resulted in a single phase during partitioning, in which case it was diluted to the extent required to produce two liquid phases (see Experimental Section). In cases where a triphase was observed or a large quantity of salt precipitated, partitioning was invariably poor, and the temperature was increased until a clean biphase was attained. While

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Table 2. Partitioning of Chlorouridine 1 between 2-MeTHF/DME (2:1 v/v, 10 mL/g) and Aqueous Salt Solutions (5 mL/g)[c]

Category	Salt	D_m	Salt Conc. (g/mL)	D_{norm}
Halide Salts	none	0.80		
	LiCl	0.15	0.42	−4.5
	NaCl	5.7	0.25	7.1
	KF	decomposition		
	KCl	3.6	0.24	5.3
	NH_4Cl	2.5	0.24	3.8
	$CaCl_2$	0.52	0.47	−1.4
	NaBr	0.80	0.55	−0.4
	NaI	0.10	0.88	−2.6
Carbonate Salts	K_2CO_3	decomposition		
	$KHCO_3$	3.2	0.23	5.1
Nitrogen Oxide Salts	$NaNO_2$	5.2	0.47	3.5
	$NaNO_3$	1.9	0.50	1.3
Halide Oxyanion Salts	$NaClO_2$	8.3	0.48	4.4
	$NaClO_3$	1.0	0.54	0.0
	$NaBrO_3$	2.2	0.25	3.2
	$NaClO_4$	0.00057	0.84	−8.9
Carboxylate Salts	Na-formate	24	0.33	9.6
	NaOAc	11	0.32	7.6
	Na-glycolate	36	0.31	12
	NaOBz	0.32	0.12	−9.6
	NaOTFA	0.055	0.16	−18
	Na_2-malonate	140	0.61	8.2
	Na_2-maleate	44	0.50	7.6
	NaK-tartrate	91	0.39	12
	K_2-oxalate[a]	42	0.26	15
	K_2-squarate	3.0	0.31	3.5
	$(NH_4)_3$-citrate	80	0.61	7.3
	Li_3-citrate	57	0.29	14
	Na_3-citrate	67	0.47	9.0
	K_3-citrate	80	0.72	6.1
	Na_4-EDTA	16	0.50	5.5
Phosphorous Oxyanion Salts	NaH_2PO_2	10	0.52	4.5
	Na_2HPO_3	120	0.89	5.4
	Na_2FPO_3	86	0.19	23
	NaH_2PO_4	92	0.52	8.7
	K_2HPO_4	390	0.81	7.4
	K_3PO_4	decomposition		
Other Chaotropic Salts	KOCN	0.28	0.45	−2.9
	NaSCN	0.00085	0.60	−12
	$NaBF_4$	0.30	0.53	−2.3
	NaCN	decomposition		
	$NaN(CN)_2$	0.070	0.18	−15

Category	Salt	D_m	Salt Conc. (g/mL)	D_{norm}
Alkali Metal Sulfates	Li_2SO_4	62	0.19	22
	Na_2SO_4[a]	82	0.17	26
	$NaHSO_4$[b]	13	0.25	10
	K_2SO_4	1.2	0.09	2.0
	Rb_2SO_4	24	0.34	9.4
	Cs_2SO_4	120	0.92	5.2
Ammonium Sulfates	$(NH_4)_2SO_4$	89	0.42	11
	$(Me_4N)_2SO_4$	9.1	0.26	8.5
	$(Bu_4N)_2SO_4$	0.18	0.11	−16
Alkaline Earth Sulfates	$BeSO_4$	25	0.34	9.6
	$MgSO_4$	38	0.28	13
Transition Metal Sulfates	$VOSO_4$	17	0.61	4.6
	$Cr_2(SO_4)_3$	1.3	0.33	0.8
	$MnSO_4$	70	0.44	9.7
	$FeSO_4$	0.58	0.21	−2.7
	$Fe_2(SO_4)_3$	19	0.95	3.1
	$CoSO_4$[a]	78	0.29	15
	$NiSO_4$[a]	110	0.28	17
	$CuSO_4$	0.91	0.15	−0.6
	$ZnSO_4$	75	0.41	11
Main Group Sulfates	$Al_2(SO_4)_3$	66	0.27	16
	$Ga_2(SO_4)_3$	59	0.51	8.0
	$In_2(SO_4)_3$	37	0.73	5.0
Other Sulfur Oxide Salts	Na_2SO_3	15	0.20	14
	$Na_2S_2O_3$	80	0.47	9.4
	NaOMs	14	0.64	4.2
	NaOTs	0.37	0.12	−8.2
	$NaSO_3NH_2$	56	0.58	7.0
	$K_2S_4O_6$	0.76	0.21	−1.3
	$(NH_4)_2S_2O_6$	20	0.40	7.4
Other Oxyanions and Cyanoanions	Na_3VO_4	0.30	0.27	−4.5
	$NaVO_3$	4.0	0.15	9.4
	Na_2MoO_4	52	0.43	9.1
	Na_2WO_4	12	0.34	7.2
	Na_2SnO_3	formed an insoluble complex		
	Na_2SeO_3	100	0.57	8.1
	Na_2SeO_4	210	0.31	17
	Na_2HAsO_4	50	0.25	16
	$Na_4Fe(CN)_6$	2.3	0.16	5.3
	$K_3Fe(CN)_6$	4.5	0.27	5.5
Zwitterions	Me_3NO	11	0.36	6.7
	betaine	5.1	0.54	3.0
	glycine	7.4	0.17	12

[a]Experiment run at 35 °C. [b]Experiment run at 50 °C. [c]The solutions were 80% of saturated unless otherwise noted in the Experimental Section. (Key: green arrow = D_m > 50, yellow arrow = 1 < D_m < 50, red arrow = D_m < 1).

temperature is known to influence partitioning,[81] the magnitude should be relatively small compared to salting effects.[82] Equilibrium can theoretically be reached within minutes,[39a] but we chose to age our mixtures for 15−18 h, in case mixing was suboptimal.[39b] In the first set of experiments, **1** was partitioned between 2-MeTHF/DME (2:1 v/v, 10 mL/g) and the aqueous salt solution (5 mL/g) at 23 °C. The screening results are presented in Table 2 with data grouped by anion type. Partitioning with pure water was very poor, giving a D_m of only 0.8, and addition of sodium, potassium, or ammonium chloride salts generally afforded only modest improvements [D_m: 2.5−5.7]. In contrast, both lithium and calcium chlorides both salted-in [D_m: LiCl (0.15), $CaCl_2$ (0.52)], aligning with the general behavior of hard cations.[77a,b,78b] The use of KF as well as other salts with pK_a values >8 (NaCN, K_2CO_3, K_3PO_4) resulted in yellow slurries that signaled a base-promoted

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Table 3. Criteria for Choosing a Salting-out Agent for Scale-up

entry	salt	D_m	% 1 extracted	D_{norm}	cost	salt stability	salt reactivity
1	NaCl	5.7	85.1	7.1	low	high	low
2	NaK-tartrate	91	98.9	12	low	high	moderate
3	Na_3-citrate	67	98.5	9.0	low	high	moderate
4	Na_2FPO_3	86	98.9	23	low	moderate	moderate
5	NaH_2PO_4	92	98.9	8.7	low	high	low
6	K_2HPO_4	390	99.7	7.4	low	high	moderate
7	$Na_2S_2O_3$	80	98.8	9.4	low	moderate	high
8	Li_2SO_4	62	98.4	22	high	high	low
9	$(NH_4)_2SO_4$	89	98.9	11	low	high	high
10	**Na_2SO_4**	**82**	**98.8**	**26**	**low**	**high**	**low**

decomposition of **1**. Even in the absence of decomposition, the uridine N−H is moderately acidic (pK_a ∼ 9.25),[83] and we would be limited to salts that are not strongly basic.

One group of salts in Table 2 with significantly enhanced partitioning compared to sodium chloride was the carboxylates. Organic salts are desirable from a greenness and sustainability point of view, but they have, to date, been underutilized for aqueous workups.[84] Generally, carboxylate salts of lower MW and multiply charged proved most effective [D_m: Na_2-malonate (140) > NaK-tartrate (91) > Na_3-citrate (67) > Na_2-maleate (44) > K_2-oxalate (42) > Na-glycolate (36) > Na-formate (24) > Na_4-EDTA (16) > NaOAc (11) > K_2-squarate (3.0) > NaOBz (0.32) > NaOTFA (0.055)]. Meanwhile, changing the cation within a series of citrate salts had a relatively small effect on the partitioning [D_m: 57−80]. Phosphates, as a class of anions, were quite adept for salting-out. While K_3PO_4 was too basic, other phosphates and fluorophosphate gave excellent organic partitioning [D_m: 86−390]. Sodium salts of more diffuse anions such as iodide, cyanate, thiocyanate, tetrafluoroborate, and dicyanamide all salted-in [D_m: 0.00085−0.28] as anticipated based on literature precedent.[35,73]

Given the favorable properties of sulfates (known to salt-out strongly, highly soluble, inert), we thoroughly explored cation partners for this class of salts. The alkali metal sulfates were all proficient at salting-out [D_m: Li_2SO_4 (62), Na_2SO_4 (84), K_2SO_4 (1.2), Rb_2SO_4 (24), Cs_2SO_4 (120)], with the exception of potassium sulfate, likely due to its marginal solubility of 90 mg/mL. Ammonium sulfate was also quite effective, but switching the cation to tetramethylammonium diminished the salting-out activity and tetrabutylammonium strongly salted-in, consistent with the proposed role of dispersion forces[36] [D_m: $(NH_4)_2SO_4$ (89) > $(Me_4N)_2SO_4$ (9.1) > $(Bu_4N)_2SO_4$ (0.18)]. Transition metal sulfates afforded a wide range of distributions [D_m: 0.58−110] that did not follow any definitive trend likely due to competing effects (via infra). Organic zwitterionic species have also been characterized as kosmotropes,[85] warranting investigation into their salting-out properties. Thus, trimethylamine *N*-oxide, betaine, and glycine were examined, but all gave mediocre partitioning [D_m: 5.1−11].

When making sense of the data from Table 2 and attempting to order individual ions analogous to the Hofmeister series, there are several factors of which to be cognizant. First, salts dissolved in water are known to dissociate to different degrees depending on the specific ion pair.[86] This scenario is exaggerated for highly concentrated solutions as in the experiments presented here. Collin's "Law of matching water affinities," which provides simple rules for anticipating the extent of ion pairing, has been invoked to explain some Hofmeister effects.[87] Second, transition metal oxyanions, namely, vanadate, tungstate, and molybdate, are known to exist as various polymers in equilibrium under a wide distribution of pH and concentrations.[88] Simple salts such as NaCl, $NaClO_4$, or KSCN can even aggregate at high enough concentrations.[89] Third, aqua cations ($M(H_2O)_x$) can hydrolyze as a function of pH to form hydroxy or oxy cations and even polymeric species as in the cases for transition metal and aluminum cations.[90] Fourth, there can be specific binding between the salt and solute as discussed earlier. A strong interaction been caffeine and copper salts has been documented[91] and could be the case here for $Cr_2(SO_4)_3$, $FeSO_4$, and $CuSO_4$, which represented outliers among the sulfate salts. Finally, uridine analogues are known to self-associate in both nonpolar[92a] and aqueous solutions,[92b,c] and some salts might have specific interactions that alter this equilibrium. It is reassuring that, despite these potentially confounding issues, most salts lined up particularly well with the Hofmeister series, especially when holding the cation constant. While it would be satisfying to present a quantitative correlation with the Hofmeister series, this is not possible due to heterogeneity of the historical experiments performed on protein aggregation.

Having examined 85 salts, a number of options were identified that provided excellent partitioning. However, many of these would not be suitable for production-scale manufacturing, as additional factors need to be considered. These factors include: (1) cost and availability, (2) chemical inertness, (3) ion stability, (4) greenness, and (5) lack of toxicity. All of the salts in the list are quite inexpensive in large quantities (<$5/kg) with the exception of Li_2SO_4 (>$50/kg, Table 3, entry 8). Some salts would only have moderate stability to the acidic aqueous conditions encountered in this process: sodium fluorophosphate (Table 3, entry 4)[93] and sodium thiosulfate (Table 3, entry 7).[94] Salts containing alcohol functionality (i.e., NaK-tartrate, Na_3-citrate), or that are moderately basic (i.e., K_2HPO_4), could pose problems to chemistry in the subsequent step if some persisted and are therefore designated as having moderate reactivity (Table 3, entries 2, 3, 6). Sodium thiosulfate (Table 3, entry 7) and ammonium sulfate (Table 3, entry 9) would definitely pose problems to chemistry in the subsequent step and are designated as having high reactivity. Ultimately, the salt that possessed all of the required criteria for process implementation was sodium sulfate (Table 3, entry 10), which also provided the highest D_{norm} value of all the candidates, helping to minimize the PMI.

The Influence of Solvent Composition and Sodium Sulfate Concentration on Salting-Out. The use of sodium sulfate as a salting-out agent was complicated by its steep

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Table 4. Response of D_m to wt % aq Na_2SO_4 (5 mL/g) and % 2-MeTHF (v/v with DME, 10 mL/g) at 35 °C

	% 2-MeTHF (v/v with DME)						
wt% Na_2SO_4	25	33.3	40	50	66.6	75	100
0	single phase				1.5	1.5	3.3
5		310	270	triphase	5.5	5.9	7.4
10		440	400	190	17	12	12
12		410	380	240	31	19	15
14		680	430	390	64	33	22
16			410	280	130	48	31
18			510	380	140	70	40
20			460	510	230	120	53
22			440	410	260	160	75

solubility/temperature gradient,[95] an acute sensitivity of partitioning to salt concentration, and to the 2-MeTHF/DME ratio. To gain confidence around our choice of conditions and demonstrate robustness, we performed a matrix of 63 experiments with Na_2SO_4 concentrations ranging from 0 to 22 wt % and the relative amount of 2-MeTHF in DME (v/v) ranging from 25% to 100% (Table 4). All partitioning experiments were performed at 35 °C to minimize the potential to form triphases that were often observed at rt. The numerical data refer to the D_m values measured for the indicated salt and solvent ratio. A few trends are immediately apparent: the first being that high partitioning is favored when the percent of 2-MeTHF is lower, although if too low, phase collapse occurs. Not surprisingly, partitioning increases with higher Na_2SO_4 concentration, but unexpectedly, on the left-hand side of the table, it appears to have minimal impact.

Measuring the percent water dissolved in the organic layer provided some insight. In the entries with 14 wt % Na_2SO_4, the water content decreased from 24.5 to 8.0 wt % with decreasing DME (Figure 4). Hence, the solubility of **1** is significantly

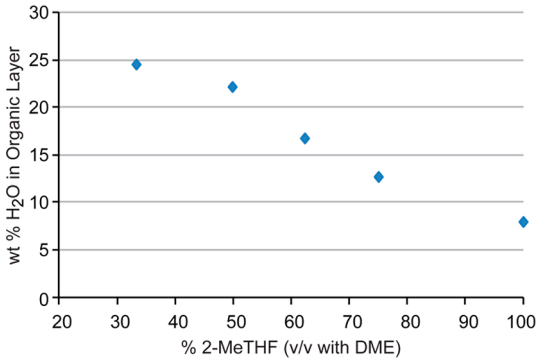

Figure 4. Water content in the organic layer as a function of the 2-MeTHF/DME ratio. Wt % Na_2SO_4 = 14.

enhanced when more water is dissolved in the organic layer, and in those experiments where Na_2SO_4 had little effect, the solubility was almost entirely dictated by solvent effects.

Given the nonlinear nature of the D_m data, the natural log values were calculated and replotted to provide a more useful representation (Figure 5). Ultimately, a 2:1 ratio of 2-MeTHF/DME and 17 wt % Na_2SO_4 (indicated by the bull's-eye) was chosen for the process to strike a balance of the amount of water dissolved in the organic layer (9−10 wt % in this case) with high partitioning (D_m = 260).

Implementing a Na_2SO_4 Aqueous Workup with Modified Hydrochlorination Conditions. Later in development, the chlorinating reagent for converting anhydrouridine **3** into chlorouridine **1** was changed from Me_3SiCl to Me_2SiCl_2, which was found to be more reactive, allowing for a lower reaction temperature and pressure. Additionally, instead of concentrating to dryness, the reaction mixture was only partially concentrated to establish a scalable process. These two changes had a markedly negative effect on the D_m, decreasing it from 80 to 12 under the optimized workup conditions using 17 wt % Na_2SO_4. We hypothesized the cause for this behavior was an increase of HCl dissolved in the process stream. HCl and sodium sulfate would be in equilibrium with sodium bisulfate and sodium chloride (Scheme 2), both of which have reduced salting-out power (see Table 2).

Titration of the second generation reaction mixture following concentration (5 mL/g) and water quenching revealed that significant HCl remained (2 equiv with respect to **3**). To quantitatively explore the effects of HCl, experiments were executed in which a crude reaction mixture of chlorouridine **1** was concentrated to dryness to remove most of the HCl. It was then partitioned between 2-MeTHF/DME (2:1, 10 mL/g) and aq Na_2SO_4 (5 mL/g) with different HCl charges. Confirming our hypothesis, a steady decline in D_m was observed for both 16 and 22 wt % aq. Na_2SO_4 with increasing HCl (Figure 6). The steep slope on the left-hand side of the graph indicated that it would be essential to have less than one equivalent of HCl and Na_2SO_4 near its saturation limit (determined to be 22−23 wt % in this solvent mixture at 35 °C) to ensure high partitioning (D_m > 30).

In a revised process (Scheme 3), the reduction of HCl to between 0.5 and 0.75 equiv was effectively achieved through a continuous constant-volume distillation with dry DME. The Na_2SO_4 charge during the workup was also increased to 22 wt % to counteract the presence of HCl. When implemented, this new workup consistently gave D_m's ranging between 40 and 50 while effectively removing HCl and enough DMF to ensure high recovery in the crystallization. The presence of 9−10 wt % water in the postworkup stream was addressed by azeotropic distillation with either MIBK or ethyl acetate, and chlorouridine **1** could then be crystallized when the water content and DME reached sufficiently low levels. Residual salt levels were typically under ~0.5 wt % in the isolated solids, with even lower amounts if a carbon treatment was performed prior to crystallization.

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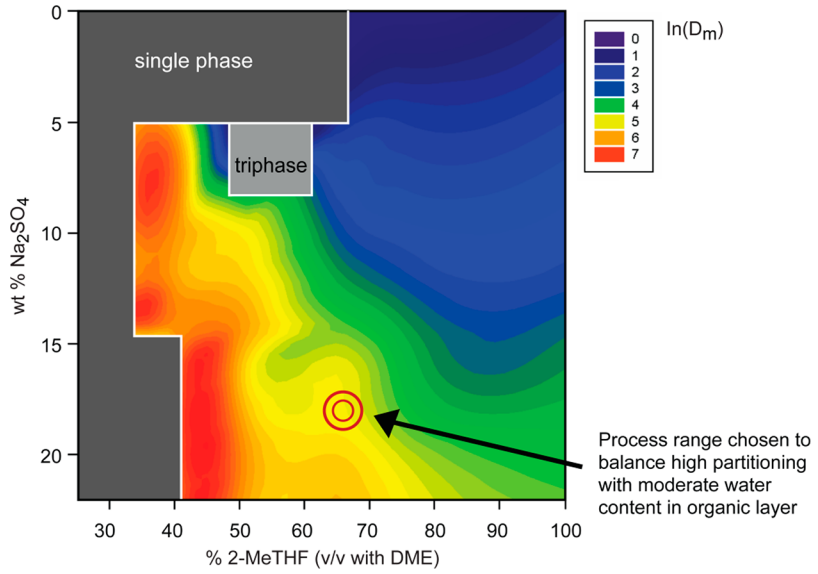


Figure 5. Contour plot of the ln(D_m) response to wt % Na_2SO_4 (5 mL/g) and % 2-MeTHF (v/v with DME, 10 mL/g) at 35 °C.

Scheme 2. Equilibrium of HCl/Na_2SO_4 with NaCl/$NaHSO_4$ and Effect on Partitioning 1

HCl + Na_2SO_4 ⇌ NaCl + $NaHSO_4$

D_m=82 D_m=5.7 D_m=13

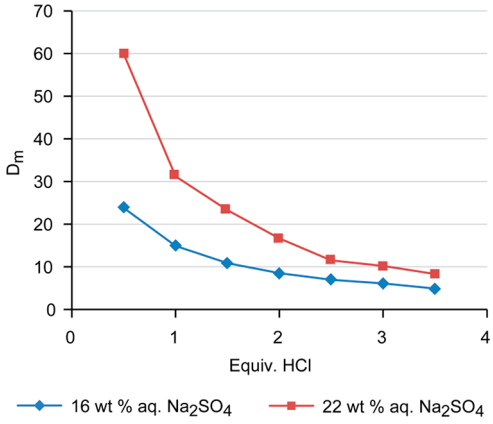
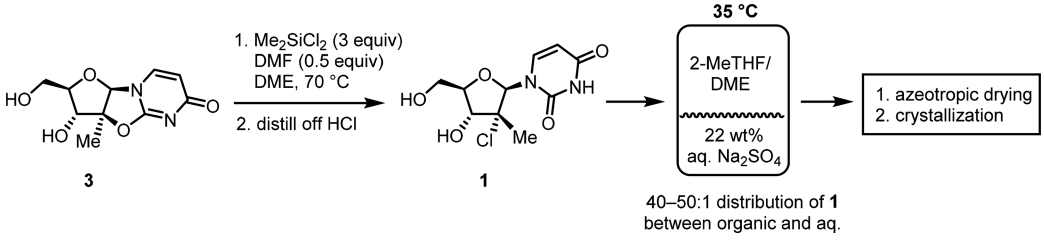


Figure 6. Partitioning of **1** between 2-MeTHF/DME (2:1 v/v, 10 mL/g) and aqueous solution (5 mL/g) as a function of HCl quantity at 35 °C.

CONCLUSION

In summary, we have presented general principles underlying specific ion effects that impact liquid–liquid partitioning and offered practical guidance on salt choice. Known trends for specific ion effects were confirmed with nucleoside **1**, and the effects of additional salts were explored. More specifically, the following anion series (while holding the cation constant: Na^+) was established in order of high to low D_{norm} values: SO_4^{2-} (26) > FPO_3^{2-} (23) > SeO_4^{2-} (17) > $HAsO_4^{2-}$ (16) > SO_3^{2-} (14) > glycolate$^-$ (12) > HSO_4^- (10) > formate$^-$ (9.6) > $S_2O_3^{2-}$ (9.4), VO_3^- (9.4) > MoO_4^{2-} (9.1) > citrate^{3-} (9.0) > $H_2PO_4^-$ (8.7) > malonate^{2-} (8.2) > SeO_3^{2-} (8.1) > maleate$^-$ (7.6), OAc^- (7.6) > WO_4^{2-} (7.2) > Cl^- (7.1) > $SO_3NH_2^-$ (7.0) > $EDTA^{4-}$ (5.5) > HPO_3^{2-} (5.4) > $Fe(CN)_6^{4-}$ (5.3) > $H_2PO_2^{2-}$ (4.5) > ClO_2^- (4.4) > OMs^- (4.2) > NO_2^- (3.5) > BrO_3^- (3.2) > NO_3^- (1.3) > ClO_3^- (0) > Br^- (−0.41) > BF_4^- (−2.3) > I^- (−2.6) > VO_4^{3-} (−4.5) > OTs^- (−8.2) > ClO_4^- (−8.9) > OBz^- (−9.6) > SCN^- (−12) > $N(CN)_2^-$ (−15) > $OTFA^-$ (−18). Of note, several of the salts (e.g., Na_2-malonate: D_m = 142.6, conc = 0.61 g/mL, D_{norm} = 8.2) have such high solubility that, even though their normalized numbers are moderate, very concentrated solutions can still salt-out quite effectively. These partitioning data were leveraged to develop an efficient salting-out extraction with a mass distribution ranging between 40 and 50 (org/aq) using sodium sulfate. A complex response of partitioning in relationship to multiple parameters (salt concentration, phase composition, presence of other ions) highlights the critical need for high-throughput methods to measure partitioning, particularly ones that are capable of determining phase volume ratios.[96] The PMI of the process was not reduced significantly due to the extra constant-volume distillation required to remove HCl from the mixture. However, this trade-off should not be generally needed for other

Scheme 3. Revised Process: Use of Me_2SiCl_2, Distillation to Remove HCl, Use of 22 wt % Na_2SO_4 for the Workup



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processes that do not have residual acids or when salts of higher buffering capacity like K_3PO_4 can be used in the extraction.

Although relative salting effects were only established for a single compound, our findings are expected to be general based on parallel trends to the extensive solubility studies reported by others and the generality of the Hofmeister series. In view of the frequency that chemists encounter difficult extractions, we wholeheartedly recommend expanded use of salting-out liquid−liquid extraction, particularly with salts that are consistent with green chemistry principles.[97] At least in the context of process development, Na_2SO_4 has been underutilized in salting-out extractions,[98] but we highly recommend increased use based on cost, efficiency, and chemical inertness considerations. In a broader context, and in view of the extensive literature available, we recommend testing a particular set of salts in addition to NaCl when significant aqueous losses are encountered during a workup: K_3PO_4, $K_4P_2O_7$ (potassium pyrophosphate), K_2HPO_4, NaH_2PO_4, Na_2FPO_3, K_2CO_3, NaOH, $(NH_4)_2SO_4$, Na_2SO_4 (at 30−40 °C to increase the amount that can dissolve in water), Na_3-citrate, NaK-tartrate, and Na_2-malonate. These salts should always be tested at high concentrations, and interpretation should account for acid−base equilibria, solute stability, and any potentially interfering ions in the mixture.

EXPERIMENTAL SECTION

General. *Reagents and Materials.* The following reagents were purchased from Sigma-Aldrich: chlorotrimethylsilane (≥99%, purified by redistillation), 2-methyltetrahydrofuran (≥98.5%, ReagentPlus, contains 150−400 ppm BHT as stabilizer), 4-methyl-2-pentanone (≥99%), ethyl acetate (99%, ReagentPlus), dimethyldichlorosilane (≥99.5%), *N*,*N*-dimethylformamide (99.8%, anhydrous), lithium chloride (≥99.0%), potassium chloride (≥99%), sodium bromide (≥99.0%), sodium iodide (99.5%), potassium carbonate (≥98%), potassium bicarbonate (99.7%), sodium nitrite (≥97.0%), sodium nitrate (ACS reagent), sodium formate (≥99%), sodium acetate (99+%), sodium benzoate (99%), sodium trifluoroacetate (98%), potassium oxalate monohydrate (≥98.5%), sodium malonate dibasic (≥97.0%), sodium maleate dibasic (≥98.0%), potassium sodium L-tartrate tetrahydrate (≥99.5%), ammonium citrate tribasic (98%), lithium citrate tribasic tetrahydrate (≥99.5%), sodium citrate tribasic dihydrate (≥99%), potassium citrate tribasic monohydrate (≥98%), ethylenediaminetetraacetic acid tetrasodium salt hydrate (98%), potassium phosphate tribasic (≥98%), potassium phosphate dibasic (≥98.5%), sodium phosphate monobasic (≥99.0%), sodium hypophosphite hydrate, sodium cyanide (97%, reagent grade), sodium thiocyanate (98%), sodium chlorite (80%, tech.), sodium chlorate (≥99%), sodium perchlorate monohydrate (98%), sodium tetrafluoroborate (98%), sodium dicyanamide (96%), sodium *p*-toluenesulfonate (95%), lithium sulfate (≥98.5%), sodium bisulfate (99%), potassium sulfate (≥99%), cesium sulfate (≥99.5%), ammonium sulfate (≥99.0%), tetramethylammonium sulfate hydrate, tetrabutylammonium sulfate (50 wt % in water), beryllium sulfate tetrahydrate (≥99.0%), manganese(II) sulfate monohydrate (99.8%), nickel(II) sulfate hexahydrate (99%), iron(II) sulfate heptahydrate (≥99%), iron(III) sulfate hydrate (97%), zinc sulfate heptahydrate (≥99.0%), copper(II) sulfate pentahydrate (≥98.0%), sodium sulfite (≥98.0%), sodium thiosulfate (99%), potassium hexacyanoferrate(III) (≥99.0%), sodium selenate (≥98.0), sodium selenite (99%), indium sulfate (≥98.0%), vanadium(IV) oxide sulfate (97%), gallium(III) sulfate hydrate (≥99.99%), tin(II) sulfate (≥95%), potassium tetrathionate (≥98%), sodium stannate trihydrate (95%), sodium molybdate dihydrate (≥99.5%), sodium tungstate dihydrate (99.0%), sodium arsenate dibasic heptahydrate (≥98%), glycine (≥99%), betaine (≥98%), trimethylamine *N*-oxide (98%), and 3,4-dihydroxy-3-cyclobutene-1,2-dione (99%). The following reagents were purchased from Acros: 1,2-dimethoxyethane (99+%, extra pure, stabilized with BHT), sodium methanesulfonate (99%), sodium bromate (≥99%), and potassium fluoride (≥99%). The following reagents were purchased from TCI: sodium sulfamate (>98.0%). The following reagents were purchased from Strem: rubidium sulfate (99.8%) and sodium fluorophosphate (94%). The following reagents were purchased from Alfa Aesar: chromium(III) sulfate hydrate (reagent grade), sodium hexacyanoferrate(II) decahydrate (99%), sodium metavanadate (96%), potassium cyanate (97%), and sodium glycolate (97%). The following reagents were purchased from Fisher: ammonium chloride (USP/FCC), calcium chloride (anhydrous), sodium vanadate (laboratory grade), sodium sulfate (certified ACS), sodium chloride (biological, certified), magnesium sulfate (certified), and cobalt(II) sulfate heptahydrate (99.9%). The following reagents were ordered from Riedel-de Haen: sodium phosphite dibasic pentahydrate (≥98%). The following reagents were ordered from Pfaltz & Bauer: aluminum sulfate octahydrate (98%). Potassium squarate was prepared by reacting 2 equiv of KOH with squaric acid in water followed by concentration. Ammonium dithionate was prepared according to the method of Gernon and Bodar.[99]

HPLC Analysis. Concentration assays for compounds **1** and **3** were carried out using an Agilent 1290 Infinity UPLC system. Column: Atlantis T3, 3 μm 4.6 × 150 mm; flow rate = 1.5 mL/min; 12.0 min runs; solvent system: MeCN and H_2O + 0.1% H_3PO_4, gradient: 0% MeCN from 0 to 2.0 min, 10% MeCN from 2.0 to 6.0 min, 10−95% MeCN from 6.0 to 8.0 min, and 95% MeCN from 8.0 to 12.0 min. The organic components were analyzed with a DAD detector at 210 nm wavelength.

1-((2R,3R,4R,5R)-3-Chloro-4-hydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (1). TMSCl Method. A 1 L Parr hybrid pressure vessel with a glass body, tantalum-coated stainless steel fittings, Hastelloy C-22 overhead stirrer, and headplate [**Caution: Due to the extreme corrosivity of this reaction, no stainless steel or lower grade Hastelloy parts (e.g., C-276) should be exposed**] was charged with DME (300 mL), anhydrouridine **3** (30.0 g, 125 mmol), and DMF (19.34 mL, 250 mmol). The apparatus was inerted by three pressure-venting cycles and then charged with TMSCl (51.1 mL, 400 mmol) resulting in a small exotherm. The mixture was heated to between 88 and 92 °C and aged for at least 18 h. The disappearance of solids indicated the reaction was complete, and HPLC analysis was used to verify the consumption of **3**; the reaction mixture was then cooled to rt.

Me_2SiCl_2 Method. A three-neck round-bottom 1 L flask with reflux condenser and overhead stirrer was charged with DME (300 mL), anhydrouridine **3** (30.0 g, 125 mmol), and DMF (4.84 mL, 62.4 mmol). The apparatus was flushed with nitrogen and then charged with dichlorodimethylsilane (45.6 mL, 375 mmol) resulting in a small exotherm. The mixture was stirred until the solids dissolved and then heated to between 68 and 72 °C. The solution was aged for at least 8 h, and once the

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consumption of **3** was verified, the solution was cooled to rt. The solution was concentrated to 7 mL/g and then distilled under vacuum (150−200 Torr) at a constant volume, [**Caution: HCl vapors should be trapped appropriately**] replenishing with dry DME (300 mL total) while maintaining the internal temperature between 40 and 50 °C. The solution was concentrated to 6 mL/g and then cooled to rt, followed by the slow addition of water (150 mL, caution: exotherm) and Na_2SO_4 (42.3 g). The mixture was vigorously stirred at 35 °C for 30 min, then let to settle, and the aqueous layer was discarded. Water was again added (60 mL) followed by Na_2SO_4 (17.9 g), and the mixture was vigorously stirred at 35 °C for 30 min, then let to settle; the aqueous layer was discarded. Azeotropic distillation with dry MIBK or ethyl acetate could effectively remove the water. Once the water and DME levels reached a sufficiently low level, **1** could be crystallized.

Preparation of 80% Saturated Salt Solutions. Saturated salt solutions were prepared by stirring an excess of the salt in deionized water overnight at ambient temperature (23−25 °C), then filtering through a disposable polypropylene filter with 10 μm polyethylene frit. Eight parts of the resulting solution was diluted with two parts DI water to obtain an 80% saturated solution. These solutions were stored in 40 mL scintillation vials at ambient conditions except for the following solutions which were stored at −20 °C in the freezer due to limited stability: $SnSO_4$, $K_2S_4O_6$, $FeSO_4$. The concentrations were determined from literature solubility values (at saturation) or measured by titration (see Supporting Information). Solution densities were measured by addition of 1.0 mL from a calibrated pipet into a glass vial and recording the weight change. For literature values reported in wt/wt, they were converted to wt/v through a density measurement. Aqueous solutions of $(Bu_4N)_2SO_4$, NaOBz, NaOTs, and NaOTFA were prepared at the concentrations indicated in Table 2 because 80 wt % solutions gave single phases in partitioning experiments.

Liquid−Liquid Partitioning Experiments. A solution of the crude reaction mixture (from the *TMSCl method* above, 30.0 g basis, 125 mmol) was concentrated to dryness by rotary evaporator [**Caution: HCl vapors should be trapped appropriately**] and then reconstituted with 2-MeTHF (180 mL) and DME (90 mL) to provide a bench-stable stock solution. An 8 mL vial with magnetic stirbar was charged with the organic solution (4.3 mL) and the salt solution (2.0 mL). The mixture was vigorously stirred for 15−18 h, and then the phases were let to separate for 10−15 min. If biphasic, the heights of the two phases were measured with a ruler. If triphasic, the mixture was heated to higher temperatures in 15 °C increments with an aluminum heating block until it became biphasic. In some cases, only a single phase was obtained, and a D_m could not be measured. The layer heights were then converted to volumes by a calibration. 500 μL aliquots (or less if the layer was too small) of each layer were diluted into individual 25 mL volumetric flasks and diluted with methanol. Concentrations of each layer were determined by HPLC using a calibration curve.

HCl Titration. Residual HCl content was determined by accurately measuring 500 μL of the chlorouridine solution into ∼100 mL of 4:1 methanol/water. The solution was then titrated with 0.1 N NaOH (aq) using a Metrohm (Herisau, Switzerland) 905 Titrando titrator operated by Metrohm Tiamo software package (autotitration based on pH).

Ammonium Citrate Titration. Ammonium citrate concentration was determined by accurately weighing 100 μL of the analyte solution into ∼100 mL of water followed by autotitration using a Metrohm Titrando (see above) based on pH (titrated with HCl). The starting pH was ∼7.5.

ICP-MS Titration. Samples were dissolved and digested with a nitric acid solution on a hot plate. The resulting sample solutions were then nebulized into the core of a PerkinElmer Optima 2100 DV inductively coupled plasma (ICP), and the analyte species (Na, K, S, and V) were then detected and quantitated with an optical emission spectrometer (OES), measuring the intensity of radiation emitted at the element-specific, characteristic wavelength from thermally excited analyte atoms or ions. Intensity measurements were converted to elemental concentration by comparison with calibration standards.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.oprd.7b00197.

Raw concentration data on partitioning experiments and information relating to salt solutions (XLSX)

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The authors declare no competing financial interest.

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