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Accelerated Koenigs−Knorr Glucuronidation of a Deactivated Nitrophenol: Unveiling the Role of Polyamine Additive 1,1,4,7,10,10-Hexamethyltriethylenetetramine[1] through Design of Experiments

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1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTTA) emerged from a limited parallel screening of selected polyamines as the most appropriate additive for an especially problematic Koenigs−Knorr glucuronidation. This initial finding rapidly evolved into a reliable and high-yielding procedure through the use of two sets of experimental designs. The detailed effect of the stoichiometry of reagents and the amount of amine additive on reaction yield was elucidated. The complexity of the response surface for product yield, described by a third-order polynomial equation, together with ancillary kinetic experiments evidenced the multiple role of HMTTA in the present glucuronidation process.

Introduction

In the endoplasmic reticulum of body cells, in particular hepatocytes, UDP-glucuronosyl transferases (UDPGTs) catalyze highly effective glucuronidation of endogenous substrates as well as xenobiotics, thus playing a central role in the mechanisms of detoxification during "phase 2" metabolism.

Disappointingly, chemists often compete inadequately with nature's efficiency when it comes to performing the same transformation outside the body.[2] Reasons for this are envisaged especially in the electron-withdrawing characteristics of the 5-alkoxycarbonyl group in the glycosyl donor, which destabilizes the incipient C-1 cation, often resulting in the prevalence of undesired deactivation pathways, e.g., ortho ester formation, acyl transfer to the aglycon, or eliminations of C-1 or C-4 substituents.

Notwithstanding the steadily increasing number of ingenious modifications or alternatives to the most common glucuronidation methods,[3] the delivery of this sugar moiety to alcohols or phenols remains challenging and far from general, often requiring extensive ad hoc optimization.

In this light, the technique of design of experiments (DOE) stands as a precious tool for the effective investigation of an appropriate "experimental space" as delimited by the low and high values of the reaction variables (factors) under study.[4] By virtue of a DOE multivariate approach, the influencing variables and, appealingly, their interactions are easily revealed. The statistical nature of the method allows a precise quantification of the effect of significant factors on a given response and, through the generation of inferential models, furnishes the experimentalist an optimum set of reaction conditions.

Results and Discussion

In the course of a recent research project, it became necessary to synthesize the glucuronide **1a** of BIMC-0576

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(1) There is no clear preference in the literature for 1,1,4,7,10,10-hexamethyltriethylenetetramine designation as different acronyms are in use. "hmteta": (a) Baxter, I.; Drake, S. R.; Hursthouse, M. B.; McAleese, J.; Malik, K. M. A.; Michael, D.; Mingos, P.; Otway, D. J.; Plakatouras, J. C. *Polyhedron* **1998**, *17*, 3777. "hmten": (b) Darr, J. A.; Poliakoff, M.; Li, W−S.; Blake, A. J. *J. Chem. Soc., Dalton Trans.* **1997**, *17*, 2869. "hmtt": (c) Shintani, N.; Kotaki, J.; Sone, K.; Fukuda, Y. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 784. "Me6trien": (d) Drumhiller, J. A.; Montavon, F.; Lehn, J.; Taylor, R. W. *Inorg. Chem.* **1986**, *25*, 3751. (e) "HMTTA": Reich, H. J.; Green, D. P.; Medina, M. A.; Goldenberg, W. S.; Gudmundsson, B. Ö.; Dykstra, R. R.; Phillips, N. H. *J. Am. Chem. Soc.* **1998**, *120*, 7201. Here we prefer the adoption of this latter abbreviation for consistency with the other acronyms used in the present paper.

(2) For a review, see: Stachulski, A. V.; Jenkins, N. J. *Nat. Prod. Rep.* **1998**, 173.

(3) (a) Stachulski, A. V. *Tetrahedron Lett.* **2001**, *42*, 6611 and references therein. See also: (b) Rukhman, I.; Yudovich, L.; Nisnevich, G.; Gutman, A. L. *Synthesis* **2000**, *9*, 1241. (c) Scheinmann, F.; Stachulski, A. V.; Ferguson, J.; Law J.-L. WO patent 00/78764 A1, 2000. (d) Vaccaro, W. D., Sher, R.; Davis, H. R., Jr. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 35. (e) Badman, G. T.; Green, D. V. S.; Voyle, M. *J. Organomet. Chem.* **1990**, *388*, 117.

(4) (a) Anderson, M.; Whitcomb, P. J. *DOE simplified: Practical Tools for effective Experimentation*; Productivity Inc.: Portland, OR, 2000. (b) Montgomery, D. C. *Design and Analysis of Experiments*; Wiley: New York, 2001. (c) Carlson, R. *Design and Optimization in Organic Synthesis*; Elsevier: New York, 2000.

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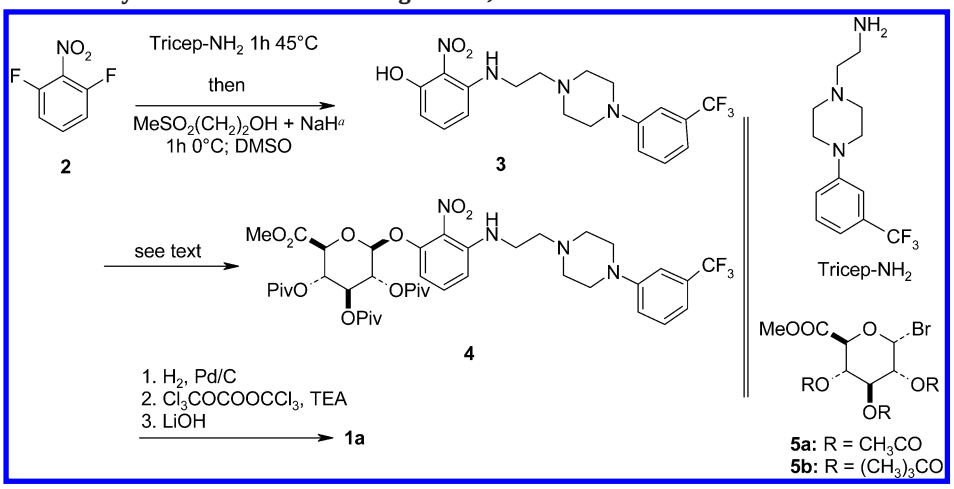
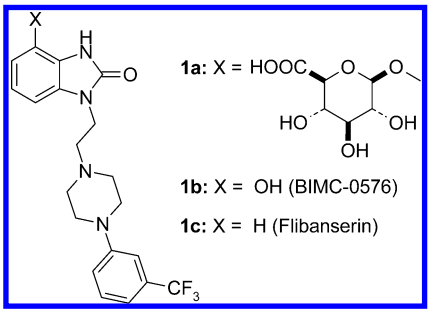
SCHEME 1. Overall Synthetic Route to Glucuronide 1a and Structural Formula for Tricep Amine (Tric-NH_2) and Differently Protected Bromo Sugars 5a,b



[a] For the first application of this reagent to the mild conversion of electron-deficient aryl fluorides to phenols, see: Rogers, J. F.; Green, D. M. *Tetrahedron Lett.* **2002**, *43*, 3585.

1b, an oxygenated metabolite of the antidepressant drug flibanserin **1c**.[5]



Initial extensive experimentation demonstrated the inapplicability of a more conservative approach,[6] i.e., direct conjugation to the corresponding aglycon **1b**. The complete unreactivity of **1b** under a wide range of glucuronyl transfer conditions forced us to a more circuitous route calling for the use of an appropriately competent glucuronyl acceptor. In this regard, the use of the *o*-nitrophenol **3** appeared promising since the beneficial effect of electron-withdrawing groups ortho positioned with respect to hydroxy groups has already been demonstrated in a number of cases.[7] Further potential benefits of this route included the easily availability of **3**. This could be very straightforwardly prepared by a new one-pot, double fluorine displacement sequence on 2,6-difluoronitrobenzene **2**. Thus, by reacting the bis-electrophile **2** with the appropriate nucleophiles in the indicated order, multigram quantities of **3** could be easily accessed. Moreover, the transformation of the protected glucuronide **4** into the desired final product **1a** could be accomplished uneventfully via nitro group reduction, cyclization to benzimidazolone, and subsequent complete deprotection of the sugar moiety (Scheme 1).[8]

Disappointingly, glucuronidation of **3** under Koenigs–Knorr conditions performed extremely poorly either employing triacetyl bromo sugar **5a** or the usually more performing Snatzke's higher homologue **5b**.[9] Although with this latter donor glucuronidation of **3** proceeded cleanly to completeness within 18 h under standard conditions (**5b** 1.2 equiv, Ag_2O 2.7 equiv in acetonitrile, in the presence of 4 Å molecular sieves) returning **4** as the only product as judged by TLC analysis, the isolated yield was as low as 3%. Considering the high potential for metal ion chelation of the aminoethylpiperazinyl moiety in **4**,[10] it was considered the chance of irreversible binding to silver ions to explain the observed mass unbalance. To test this hypothesis, the glucuronidation reaction was repeated in the presence of 10 equiv of a chelating amine, namely *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA). Interestingly, a 27% yield of glucuronide **4** could be isolated under these conditions but any attempt at further optimization by the traditional changing one-variable-at-a-time (OVAT) approach did not meet with substantial success.

Under these circumstances, it was considered preferable to adopt the more systematic DOE tactic. Our initial optimization approach was to examine the effect of various amines and silver sources on reaction yield and purity. To this end, a set of five amine additives (see Table 1) was selected on the basis of different complexing ability[11] and basicity,[12] each amine being tested in the presence of two silver sources: Ag_2O and Ag_2CO_3. The

(5) Borsini, F.; Brambilla, A.; Ceci, A.; Cesana, R.; Giraldo, E.; Ladinsky, H.; Monferini, E.; Turconi, M. *Drugs Future* **1998**, *23*, 9.
(6) Paleari, F.; Rondina, F. Unpublished results.
(7) Alkyl salicylates (a, b) or *ortho*-nitro phenols (c, f) are superior substrates in Koenigs–Knorr glucuronidations due to hydroxyl activation via intramolecular H-bonding. For pertinent examples, see: (a) Lunsford, C. D.; Murphey, R. S. *J. Org. Chem.* **1956**, *21*, 580. (b) Rossignol, J.-F.; Ayers, M. S. US Patent 5,952,622, 1999. (c) Kiss, J.; Burkhardt, F. *Carbohydr. Res.* **1970**, *12*, 115. (d) Roffler, S.; Chern, J.-W.; Leu, Y.-L. US Patent 6,043,367, 2000. (e) Jacquesy, J.-C.; Gesson, J.-P.; Monneret, C.; Mondon, M.; Renoux, B.; Florent, J.-C.; Koch, M.; Tillequin, F.; Sedlacek, H. H.; Gerken, M.; Kolar, C.; Gaudel, G.; Bosslet, K.; Czech, J.; Hoffman, D.; Seemann, G.; Schorlemmer, H.-U.; Dickneite, G. US Patent 5,561,119, 1996. (f) Goto, K.; Nakamura, S.; Morioka, Y.; Kondo, M.; Naito, S.; Tsutsumi, K. *Chem. Pharm. Bull.* **1996**, *44* (3), 547.
(8) See the Supporting Information.
(9) Vlahov, J.; Snatzke, G. *Liebigs Ann. Chem.* **1983**, 570.
(10) Stable complexes between silver (I) ions and polyamines have been observed in acetonitrile [(a) Grzejdziak, A. *Pol. J. Chem.* **1994**, *68*, 1395] and DMSO: (b) Comuzzi, C.; Novelli, V.; Portanova, R.; Tolazzi, M. *Supramolecular Chem.* **2001**, *13*, 455.

TABLE 1. Amine Additives and Silver Sources Parallel Screening for Glucuronidation of 3[a]

		in situ yield of **4** (%)	
expt	amine additive[b]	Ag_2O	Ag_2CO_3
1−2	DIPEA	0	0
3−4	TMEDA	24.8	29.3
5−6	DIPEDA	26.1	15.2
7−8	DMEDA	0	0
9−10	HMTTA	40.6	38.6

[a] 10 mg scale, 1.5 equiv of bromo sugar **5b**, 2.7 equiv of silver source, 10 equiv of amine additive, acetonitrile (0.15 mL), 6 h. [b] DIPEA, diisopropylethylamine; TMEDA, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; DIPEDA, *N*,*N*′-diisopropylethylenediamine; DMEDA, *N*,*N*′-diimethylethylenediamine; HMTTA, 1,1,4,7,10,10-hexamethyltriethylenetetramine.

10 experiments were conducted on a small-scale (10 mg) parallel format with 1.5 equiv of bromo sugar **5b** and the appropriate silver source (2.7 equiv) in acetonitrile at rt in the presence of 10 equiv of amine additive. Analysis of in situ yields (Table 1), as determined by HPLC (external standard) after 6 h, clearly suggested a strong positive correlation between coordinating ability of the amine additive and solution yield, as evident by the substantially better result (average in situ yield 39.6%) obtained in the presence of tetradentate amine: 1,1,4,7,-10,10-hexamethyltriethylentetramine (HMTTA).[13] Two other considerations appeared meaningful at this point. First, use of strongly basic conditions should be limited.[14] Thus, the chelating yet unhindered and highly basic DMEDA performed (in situ yield 0%) much worse than TMEDA or DIPEDA (27.0% and 20.6% yield respectively). Second, the choice of silver source did not appear to significantly affect yields.[15]

On the basis of these findings and considering the results from the attempted OVAT optimization, a list of seven expected critical parameters (see Table 2) in this reaction was set up to be studied in a two-level factorial design. Their number and the likely interactions between them convinced us to apply a fractional factorial design with low resolution, i.e., a 2^{7-4} resolution III design. Such "saturated" designs ($k + 1$ runs, with k factors to be studied) allow relevant effects to be discovered with a minimum number of experimental trials,[16] yet their highly fractional nature produces critical confusion between effects. In the specific "resolution III" case, main effects are confused ("aliased") with one or more two-factor interactions; that is, the effect due to a specific main factor may be additionally due to the combined effects of all or each of these higher order interactions. Although in many cases this low resolution can still be adequate, when needed the appropriate duplication of experimental runs will resolve (de-alias) significant effects. Thus, a complete (or partial) "foldover" of a resolution III design makes it resolution IV, where main effects are now aliased only with highly unlikely three- or higher-order interactions. In the present case, a set of eight experiments was generated,[17] and three center points replicates were added to provide a measure of pure experimental error and to check for curvature, i.e. non linearity of the response. The 11 experiments (50 mg scale) were performed and analyzed in random order to measure the in situ yield of the desired glucuronide **4**. The experimental matrix and the responses obtained under each factorial combination are depicted in Table 3.

TABLE 2. Variables Considered and Levels Used in the 2^{7-4} Factorial Design (Resolution III)[a]

variables under study		(−)	0	(+)
A	precomplex. time[b] (min)	0	30	60
B	reaction time[c] (h)	2	4	6
C	Ag_2CO_3 (equiv)	1.5	2.6	3.8
D	HMTTA (equiv)	1.5	7.1	12.6
E	**5b** (equiv)	1.5	2.2	3
F	4 Å mol sieves (mg)	0	50	100
G	solvent (mL)	0.5	1	1.5

[a] Constant factors: temperature (25 °C), stirring speed (1000 rpm). [b] Stirring time before addition of bromo sugar **5b** and phenol **3**. [c] Stirring time after addition of **5b** and **3**.

TABLE 3. 2^{7-4} Factorial Design:[a] Experimental Matrix and Measured Response[b]

	factor settings							
expt	A	B	C	D	E	F	G	yield of **4** (%)
1	−	−	−	+	+	+	−	14.7
2	+	−	−	−	−	+	+	19.5
3	−	+	−	−	+	−	+	24.4
4	+	+	−	+	−	−	−	11.2
5	−	−	+	+	−	−	+	34.2
6	+	−	+	−	+	−	−	83.2
7	−	+	+	−	−	+	−	56.5
8	+	+	+	+	+	+	+	55.4
9	0	0	0	0	0	0	0	50.2
10	0	0	0	0	0	0	0	43.2
11	0	0	0	0	0	0	0	50.5

[a] 50 mg scale, 25 °C, stirring speed 1000 rpm; the experiments were performed in random order. [b] Measured by HPLC using an external reference standard.

The normal plot of the effects for the in situ yield of **4** (Figure 1) revealed that, among the seven experimental

(11) The affinity of screened amines for metal ions is expected to increase in the following order: DIPEA < TMEDA < DMEDA ∼ DIPEDA < HMTTA. Tertiary amines are poorer σ−donors and, therefore, also poorer ligands than the corresponding primary and secondary amines; for a thorough discussion on the effect of N-alkylation on the stability of polyamine−metal complexes, see ref 12d.

(12) In water, the pK_a values for the screened amines are as follows: (a) DIPEA = 11.0. Ramirez, F.; Marecek, J. F. *Tetrahedron* **1981**, *35*, 1581. (b) DIPEDA = 10.40, 7.59 and DMEDA = 10.29, 7.47. Basolo, F.; Murmann, R. K.; Chen, Y. T. *J. Am. Chem. Soc.* **1953**, *75*, 1478. (c) TMEDA = 9.14, 5.90. Gustafson, R. L.; Martell, A. E. *J. Am. Chem. Soc.* **1959**, *81*, 525. (d) HMTTA = 9.23, 8.47, 5.36, 1.68. Golub, G.; Cohen, H.; Paoletti, P.; Bencini, A.; Messori, L.; Bertini, I.; Meyerstein, D. *J. Am. Chem. Soc.* **1995**, *117*, 8353.

(13) While only one (mixed) complex between HMTTA and Ag (I) cations has been observed and fully characterized (ref 1b), several stable complexes of this polydentate amine and different metallic ions are known. For diverse examples, see: [Cu] ref 1d, 12d and (a) Becker, M.; Heinemann, F. W.; Knoch, F.; Donaubauer, W.; Liehr, G.; Schindler, S.; Golub, G.; Cohen, H.; Meyestein, D. *Eur. J. Inorg. Chem.* **2000**, *4*, 719. [Gd and Y] ref 1a. [Pd] (b) Bazzicalupi, C.; Bencini, A.; Cohen, H.; Giorgi, C.; Golub, G.; Meyerstein, D.; Navon, N.; Paoletti, P.; Valtancoli, B. *J. Chem. Soc., Dalton Trans.* **1998**, *10*, 1625. [Ni] ref 1c.

(14) DIPEA is 10^7 times more basic in acetonitrile (pK_a 18.1) than in water (pK_a 11.1). Kelly-Rowley, A. M.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **1995**, *117*, 3438.

(15) Such a set of experiments can be regarded and analyzed as a general factorial design, where the factors (silver source and amine additive) are categorical, i.e., non-numeric, and may have a different number of levels (two and five, respectively). In this case, analysis of results indicated no relationship between type of silver source and product yield with a prob > 0.544; this indicates the observed variation in responses is very probably due to experimental noise.

(16) See refs 4a, pp 109−122, and 4b, pp 337−347.

(17) *Design Expert*, version 6.0.4, by Stat-Ease (www.statease.com) was used for generation and analysis of experimental designs.

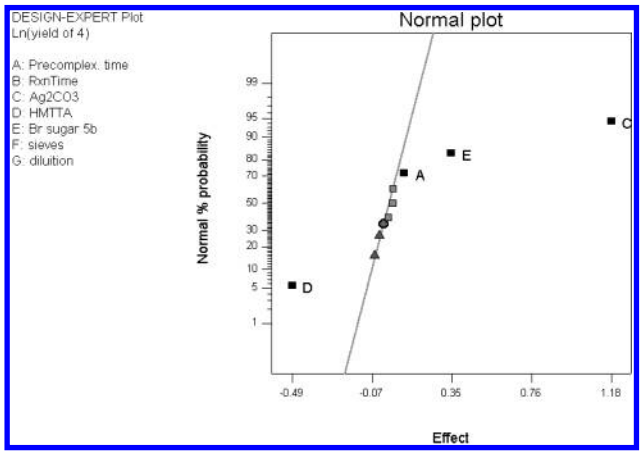


FIGURE 1. Normal pot for the screening 2^{7-4} factorial design.

variables, stoichiometry of reagents was especially important, i.e., equiv of Ag_2CO_3 (factor *C*; effect +39.85), equiv of bromo sugar **5b** (factor *E*; effect +14.08), and equiv of amine additive (factor *D*; effect −17.02). Precomplexation time between the amine additive and the silver source (factor *A*; effect +9.87) also appeared to be significant (prob = 0.099). While the possibility of precomplexation between the amine additive and Ag^+ ions could not be regarded as remote,[13] the alias structure for *A* (*A* = *A* + *BD* + *CE* + *FG*) reveals a strong correlation between factor *A* and three two-factor interactions, namely *BD*, *CE* and *FG*. In particular, the interaction between equiv of Ag_2CO_3 and equiv of bromo sugar **5b** (*CE*)[18] could be regarded especially probable also because both parents (*C* and *E*) already appear significant. Anyhow, since it was considered risky to arbitrarily dismiss the importance of precomplexation time (*A*) in favor of *CE* interaction, we considered the effect of *A* being operative together with the *CE* interaction, and no further experimentation was deemed necessary to increase design resolution at this stage. Finally, reaction time (*B*), presence of molecular sieves (*F*) and dilution (*G*) appeared marginal (P > 0.30) and their level could therefore be set to the most practical value.

Use of *A*(+*CE*), *C*, *D*, *E* led to a multiple regression model (eq 1, coded factors) with no lack of fit ($P = 0.860$) and good prediction ability ($R^2 = 0.994$, adj $R^2 = 0.989$, pred $R^2 = 0.981$, PRESS = 0.076).

$$\text{Ln(in situ yield of } \mathbf{4}) = 3.41 + 0.047A + 0.59C - 0.25D + 0.17E \quad (1)$$

As typical with fractional designs, the optimal reaction conditions are located on the very limits of the design space at Ag_2CO_3 3.8 equiv, bromo sugar **5b** 3.0 equiv, and HMTTA 1.5 equiv, where standard error is highest. In addition, a rather large curvature effect is evident, the actual averaged yield for the three center points being significantly ($P = 0.0001$) higher than the predicted value (48.0% vs 30.2% respectively), yet the low resolution of this design does not permit to establish which factor(s) is (are) causing it. These observations underscore the complexity of the system and justify the use of response surface methods to get a definitive model of the reaction.

TABLE 4. Central Composite Design[a] (CCD) and Subsequent Modifications: Factors Settings and Measured Responses[b]

	factor settings			measured responses	
	A	B	C	yield of residue	
expt	equiv of HMTTA	equiv of Ag_2CO_3	equiv of sugar **5b**	**4** (%)	**3** (%)
1[c]	0.7	3.7	2.1	82.8	11.4
2[c]	2.1	3.7	2.1	63.5	11.2
3[c]	0.7	5.1	2.1	81.6	5
4[c]	2.1	5.1	2.1	69.9	15.9
5[c]	0.7	3.7	2.4	87.5	12.4
6[c]	2.1	3.7	2.4	72.8	20.8
7[c]	0.7	5.1	2.4	85.1	4.8
8[c]	2.1	5.1	2.4	74.3	15.4
9[c]	0.2	4.4	2.25	62.1	3.6
10[c]	2.5	4.4	2.25	70.2	19.0
11[c]	1.4	3.3	2.25	73.9	21.6
12[c]	1.4	5.5	2.25	77.0	9.1
13[c]	1.4	4.4	2.0	71.9	14.4
14[c]	1.4	4.4	2.5	79.6	12.1
15[c,d]	1.4	4.4	2.25	71.8	12.0
16[c,d]	1.4	4.4	2.25	79.8	14.0
17[c,d]	1.4	4.4	2.25	77.6	13.9
18[c,d]	1.4	4.4	2.25	77.2	14.2
19[c,d]	1.4	4.4	2.25	76.6	12.8
20[c,d]	1.4	4.4	2.25	78.4	13.3
21[e]	0.2	4.4	2.1	75.0	3.3
22[e]	0.2	4.4	2.25	78.4	3.4
23[e]	0.2	4.4	2.4	80.7	9.2
24[e]	2.5	4.4	2.1	66.4	19.0
25[e]	2.5	4.4	2.25	72.1	18.8
26[e]	2.5	4.4	2.4	72.5	17.8

[a] 100 mg scale, 25 °C, stirring speed 1000 rpm, 60 min precomplexation time, 4 h reaction time; the experiments were performed in random order within each block. [b] Measured by HPLC using external reference standards. [c] First block. [d] Center point. [e] Second block.

A central composite design[19] (CCD) was therefore implemented in a second experimental plan. The factors under investigation (see Table 4) over the CCD five-level format were reagent stoichiometries previously found important. The selection of factors ranges under study reflected the desire to thoroughly explore the region of greatest promise as evident from the previous screening experiment. The remaining significant factor, precomplexation time between HMTTA and Ag_2CO_3, was purposefully omitted from the study to limit the number of experimental runs and was kept to its best, yet practical value of 60 min. With three factors, the proposed CCD experiment (Table 4) comprised a total of $2^3 + 2 \times 3 + 6 = 20$ experimental runs: eight factorial runs, six axial runs, and six replicates of the center point run. As before, the runs (100 mg scale) were conducted in random order in groups of five, gauging after 4 h the in situ yield of glucuronide **4** and the content of residual starting material **3**. To this initial design a second block of experimental runs was eventually added. Six runs at different bromo sugar **5b** and HMTTA stoichiometries and a constant amount of Ag_2CO_3 were separately performed at the extremes of the experimental space in order to

(18) The interaction effect between C and E is evident as follows: experiments that employ high Ag_2CO_3 and low bromo sugar **5b** stoichiometries perform in average much worst than when both factors are set to their high level (*C*+ *E*−: 40% vs *C*+ *E*+: 72%). On the other hand, low Ag_2CO_3 stoichiometry affords low and non significantly different yields, regardless of the amount of bromo sugar (*C*− *E*−: 16% and *C*− *E*+: 20%).

(19) See ref 4b, pp 456−458.

TABLE 5. ANOVA Table and Associated Statistics for Response Surface Reduced Cubic Model for Yield of 4[a]

source	sum of squares	degrees of freedom	mean squares	*F* value	prob > *F*	β_i[b]	α_i[c]
model	686.640	6	114.441	26.222	<0.0001	76.91	48.00
A	293.242	1	293.242	67.190	<0.0001	−9.58	21.63
B	6.641	1	6.641	1.522	0.233	0.70	−3.29
C	117.042	1	117.042	26.818	<0.0001	2.57	17.16
A^2	18.610	1	18.610	4.264	0.054	−0.75	−34.08
AB	16.502	1	16.502	3.782	0.068	1.44	3.13
A^3	121.418	1	121.418	27.820	<0.0001	2.51	7.82
residual	78.559	18	4.364				
lack of fit	41.219	13	3.171	0.424	0.901		
pure error	37.340	5	7.468				
corr total	765.202	24					

$S = 2.089$, $R^2 = 0.897$, adj $R^2 = 0.863$, pred $R^2 = 0.808$, PRESS = 146.59

[a] The outlier point corresponding to reaction 9 of Table 4 was not included in the model (actual value: 62.100, predicted value: 79.960, residual −17.860, Student residual −3.677, outlier *t* −5.788). [b] Estimated regression coded coefficients for in situ yield of **4**. [c] Estimated regression actual coefficients for in situ yield of **4**.

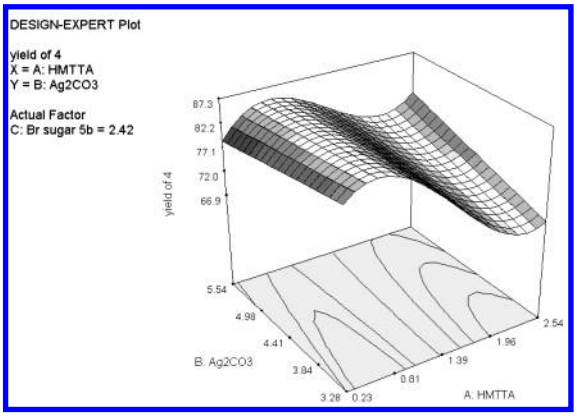


FIGURE 2. Influence of stoichiometry of Ag_2CO_3 (B) and HMTTA (A) on the in situ yield of **4** at bromo sugar **5b** equal to 2.42 equiv.

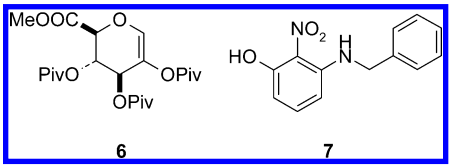
improve model predictivity in these regions. The data for glucuronide **4** yield from the first 20 experimental runs (first block) were fitted to a cubic equation ($R^2 = 0.828$, adj $R^2 = 0.748$, pred $R^2 = -0.947$, PRESS = 1526.9). As anticipated, inclusion of the results from the remaining six runs (second block) and exclusion of the strong outlier corresponding to run 9 in Table 4 substantially improved model prediction ability ($R^2 = 0.897$, adj $R^2 = 0.863$, pred $R^2 = 0.808$, PRESS = 146.6) without altering this conclusion. Analysis of variance testing (Table 5) confirmed the significance of the cubic term A^3 as well as of variables A^2, *A*, and *C* and of two-factor interaction *AB*. The statistically nonsignificant, yet hierarchically important,[20] linear term *B* was also included in the analysis to provide the model depicted in eq 2 (coded factors).

$$\text{in situ yield of } \mathbf{4} = 76.91 - 9.58A + 0.70B + 2.57C - 0.75A^2 + 1.44AB + 2.51A^3 \quad (2)$$

The three-dimensional surface plot, illustrated in Figure 2, was generated according to the cubic model and demonstrates the dependence of in situ yield of glucuronide **4** on the amounts of amine additive HMTTA (variable *A*) and Ag_2CO_3 (variable *B*) at the optimal level of bromo sugar **5b** (variable *C*). Similar plots were obtained at different concentrations of bromo sugar **5b**.

As evident from the plot, the yield of **4** correlates quite oddly with the amount of amine additive. While optimal performance in the coupling step is predicted when the concentration of HMTTA approaches 0.6−0.7 equiv, a further increase (up to ca. 1.8 equiv) of HMTTA hinders the reaction. Surprisingly enough, sufficient concentrations (ca. 2.5 equiv) of this amine additive reverse this trend and conjugation appears again promoted. The surface twist introduced by inclusion of interaction term *AB* in the model accentuates (or flattens) this behavior without nevertheless altering this basic frame.

Fundamentally, such a complex situation could underline a multiplicity of, often conflicting, roles played by the amine additive in the present glucuronidation process. Speculations on this point revealed that HMTTA might facilitate the conjugation acting: (1) as a competitive ligand for silver ions, capable of limiting the postulated irreversible bond to aminoethylpiperazinyl moiety in the starting material **3** or the reaction product **4**; (2) as an activator for the silver source, functioning as a shuttle for silver ions to be delivered into solution in a complexed, yet active form; (3) as a base: increasing the nucleophilicity of phenol **3** by deprotonation.[21] Alternatively (4), high concentration of this basic additive may hamper the conjugation by contributing to effective depletion of bromo sugar **5b** from the reaction mixture via the known dehydrohalogenation at C-1, eventually producing 2-pivaloyloxyglycal **6**.[22] As a matter of fact, analysis of the data from the modified CCD indicated a strong and significant (effect = +8.68; $P < 0.0001$, see the Supporting Information) direct relationship between variable *A*, concentration of HMTTA, and content of residual starting material **3**, supporting this latter assumption.



To substantiate the hypothesis of irreversible binding between substrates and silver ions and to gain a better

(20) The hierarchy principle dictates that internal consistency in a model containing a high-order term is promoted by inclusion of all the lower order terms that compose it.

(21) However, this effect should not be considered as pivotal since glucuronidation of potassium phenoxide of **3**, delivered only trace amount of glucuronide **4** when performed in the absence of the amine additive.

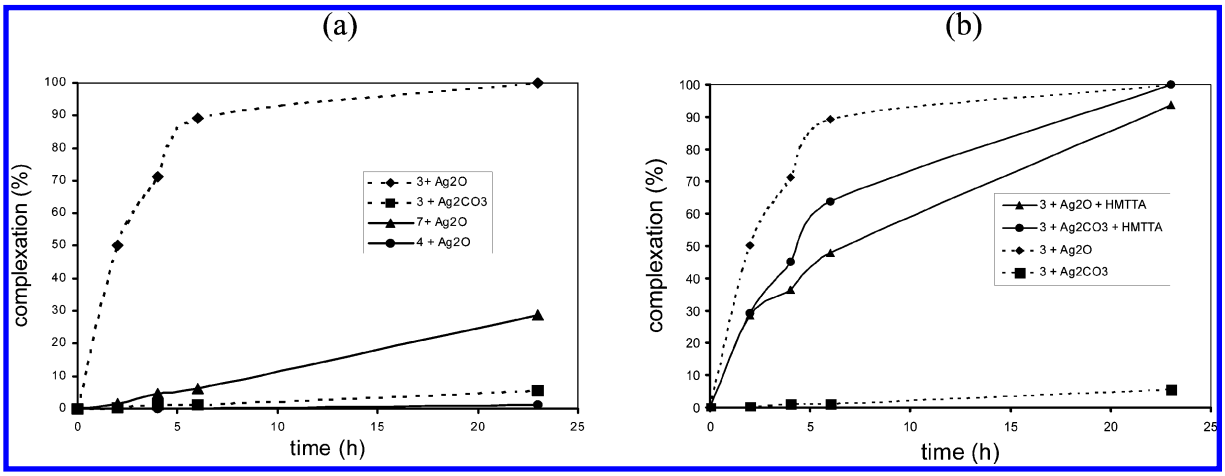


FIGURE 3. Kinetic studies for complexation of **3**, **4**, and **7** to silver ions: structure vs silver source relationship for complexation equilibria of **3**, **4**, and **7** (a); effect of excess HMTTA (10 equiv) and silver source on complexation of **3** (b).

understanding of the role of the amine additive in the glucuronidation process, a complementary set of experiments was organized. To this end, mixtures of Ag_2O (2.7 equiv) in acetonitrile were separately treated with starting material **3**, glucuronidation product **4,** and 3-benzylamino-2-nitrophenol **7**[23] and monitored at 2, 4, 6, and 24 h stirring time using toluene (or xylene) as internal standard. As the data in Figure 3a show, irreversible binding is actually operative in the case of starting material **3** since this nitrophenol was almost consumed after 6 h, and completely absent after 24 h. By contrast, reaction product **4** was not noticeably affected by the presence of silver ions, its concentration in the reaction mixture remaining constant over the same period of time. Nitrophenol **7** appeared to bind, although less efficiently than **3**, to silver ions. Clearly, while the presence of aminoethylpiperazinyl moiety remains essential group for an effective binding to silver ions it appears the actual binding site must additionally comprise the phenolic group. Surprisingly, with Ag_2CO_3 as silver source (2.7 equiv), complexation of **3** looks much less efficient, probably due to a lower solubility of this salt in acetonitrile. As for the role of amine additive on complexation equilibria (Figure 3b), it emerged that with Ag_2O the presence of HMTTA (10 equiv) thwarts the complexation of **3** thus demonstrating the ability of this tetramine to function as competitive ligand for silver ions. Oddly, in the case of Ag_2CO_3 the effect of amine additive is opposite. Here, the complexation of **3** is substantially increased by addition of HMTTA (10 equiv) suggesting the prevalence of silver salt dissolution processes over the expected silver ions scavenging action. A final series of complexation experiments at different HMTTA concentrations (Figure 4) corroborates this point. The observed nonlinear relationship between complexation of **3** and concentration of the tetramine additive suggests the coexistence of silver ion competitive ligation and silver ion solvation processes.

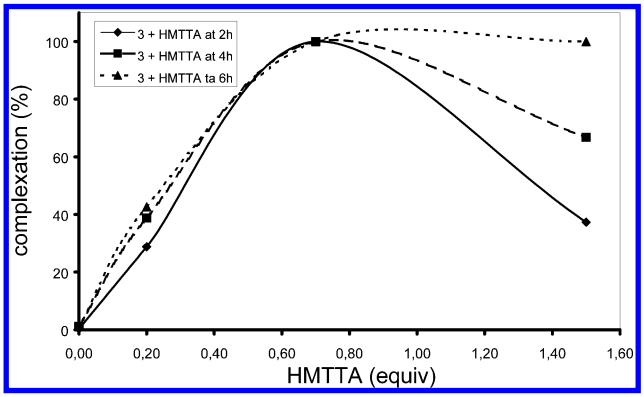


FIGURE 4. Effect of different amounts of HMTTA on complexation equilibria between **3** and Ag_2CO_3 at various reaction times.

Thus, low (up to 0.7 equiv) HMTTA concentrations progressively facilitates transport of Ag^+ in solution and subsequent complexation to the substrate. At higher concentrations, HMTTA appears to compete with **3** in the binding to silver ions and complexation of the substrate is lessened.

This subtle game of multiple and contradictory effects produces the observed complexity in the response surface describing the interplay between significant factors and glucuronidation in situ yield. Such a complex situation could hardly have been only pictured through a traditional monovariate approach lacking a homogeneous coverage of the experimental space.

Interrogation of the multivariate model in eq 2 was finally used to predict optimal conditions for glucuronidation of **3**. Thus, an 86.5% ± 2.8% in situ yield was expected by adding sequentially 1 equiv of nitrophenol **3** (100 mg scale) and 2.40 equiv of bromo sugar **5b** to a prestirred (for 1 h) mixture of Ag_2CO_3 (3.7 equiv) and HMTTA (0.70 equiv) in acetonitrile. This prediction was confirmed by conducting the glucuronidation on a 1.0 g (twice) and 3.5 g scale under these conditions. The observed in situ yields for glucuronide **4** were 86.0, 87.2 and 85.7% by HPLC analysis, and the isolated (by flash chromatography) yields were 80.6, 81, and 80% respectively. Close monitoring of the reaction progress indicated a performance peak after only 0.5 h, thus these bigger

(22) This HBr elimination is a persistent secondary process in glucuronidations of phenolates with bromo sugar **5a**: Anderson, F. B.; Leabeck, D. H. *Chem. Ind. (London)* **1960**, 967. Similarly, **5b** is completely decomposed when exposed to 1 equiv of EMDTA for 72 h producing **6** as the major product (see the Supporting Information).

(23) Prepared in 64% yield, analogously to **2**, by a one-pot reaction of 2,6-difluoronitrobenzene with benzylamine and 2-hydroxyethylmethylsulphone in DMSO (see the Supporting Information.)

scale reactions were analyzed and worked up after this time. Interestingly, under the same conditions but in the absence of HMTTA additive, no trace of reaction product **4** was evident in the reaction mixture even after 4 h.

In summary, an experimental strategy that combines a selected reagent screening and statistical design of experiments (DOE) has allowed the rapid identification of optimal conditions for an especially problematic Koenigs−Knorr glucuronidation. The isolated yield in this newly developed process was greater than 80% after 0.5 h of reaction time. These data compare extremely favorably to those experienced prior to optimization (27% after 18 h).

The chelating tetramine HMTTA initially emerged from the screening of selected (poly-)amines as the most promising candidate. Through the use of aptly designed experimental matrixes the dependence of in situ yield of glucuronide **4** from stoichiometry of HMTTA was modeled. The observed complex relationship is thought to reflect the multiplicity of roles played by this amine additive in the present process, as also suggested by a set of ancillary kinetic experiments. From the experimental data is also evident the remarkable effect of HMTTA in significantly facilitating the glucuronidation process, even when used catalytically. Thus while 0.7 equiv of HMTTA provide optimal yields of **4**, a 75−80% in situ yield was also secured by utilizing only 0.2 equiv of this additive. The generality of this method is presently under study. Preliminary results on phenol **7** and vanillin indicate analogous rate acceleration and yield enhancement as for **3** (for **7** after 1 h reaction time: 100% yield at 95% conversion with 0.7 equiv of HMTTA *vs*. 5% conversion in the absence of the additive and in the case of vanillin after 1 h reaction time: 93% yield at 95% conversion vs 33% yield at 35% conversion with no HMTTA) thus setting the basis for extending the present results to a wider range of differently substituted phenols.

Experimental Section

Methyl 3-[[2-[4-(3-Methylphenyl)piperazin-1-yl]ethyl]-amino]-2-nitrophenyl-2,3,4-tris-*O*-(2,2-dimethylpropanoyl)-β-D-glucopyranosiduronate (4). Scale-up Procedure. A mixture of Ag_2CO_3 (3.5 g, 12.7 mmol) and HMTTA (1.3 mL, 4.78 mmol) in 35 mL of CH_3CN was stirred at room temperature for 1 h. After this time, phenol **3** (3 g, 6.76 mmol), bromo sugar **5b** (8.57 g, 16.37 mmol), and 35 mL of CH_3CN were added. The reaction mixture was stirred at room temperature for 30 min and then filtered, washing the insoluble material with EtOAc (2 × 50 mL). After evaporation of the solvent under reduced pressure and silica gel purification (SiO_2 250 g; hexane/EtOAc 8/2 to 2/1), protected glucuronide **4** was obtained as a bright yellow foam (4.65 gr, 80%): mp 96.8-97.5 °C; $[\alpha]_D$ −0.7 (c = 2, $CHCl_3$); analytical HPLC t_R 22.50 min (gradient CH_3CN/phosphate buffer pH 7: 60:40 to 80:20 5 min, 80:20 20 min, postrun 7 min, flow rate 1 mL/min); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.34 (1H, t, J = 8.0 Hz), 7.23 (1H, t, J = 8.4 Hz), 7.11(1H, s), 7.07 (2H, t), 6.47 (1H, s, exchangeable), 6.46 (1H, d, J = 8.4 Hz), 6.42 (1H, d, J = 8.2 Hz), 5.44 (1H, t, J = 9.2 Hz), 5.36 (2H, m), 5.26 (1H, d, J = 7.8 Hz), 4.21 (1H, d, J = 9.9 Hz), 3.72 (3H, s), 3.28-3.26 (6H, m), 2.7-2.6 (6H, m), 1.15 (9H, s), 1.14 (9H, s), 1.13 (9H, s); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 177.0, 176.4, 176.0, 166.7, 151.3, 150.8, 143.6, 134.6, 133.0, 131.4 (q, J_{C-C-F} = 31.12 Hz), 129.5, 124.3 (q, J_{C-F} = 270 Hz), 118.7, 115.7 (d, $J_{C-C-C-F}$ = 3.75 Hz), 112.2, 107.9, 103.9, 98.9, 72.9, 71.5, 70.4, 69.0, 55.5, 52.8, 52.4 (2 × CH_2), 48.6 (2 × CH_2), 39.8, 38.7 (2 × C), 38.7, 27.1 (3 × CH_3), 27.0 (3 × CH_3), 26.9 (3 × CH_3); MS (APCI$^+$) 853 (M + H$^+$, 100). Anal. Calcd for $C_{41}H_{55}F_3N_4O_{12}$: C, 57.74; H, 6.50; N, 6.57. Found: C, 57.19; H, 6.47; N, 6.64.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra and experimental procedures for the preparation of **1a**, **3**, **4**, **6**, **7**, **9**, and **10** and HPLC analytical data for designed CCD experiment. Analysis of CCD experiment for residual starting material **3** and ANOVA table for the 2^{7-4} factorial design. This material is available free of charge via the Internet at http://pubs.acs.org.

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