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REACTION OF PYRIDINE DERIVATIVES WITH BUTYL GLYCIDYL ETHER AS A MODEL SYSTEM FOR GLYCIDYL ETHER MODIFIED AGAROSE: STRUCTURAL ASSIGNMENT BY SELECTIVE INEPT SPECTRA

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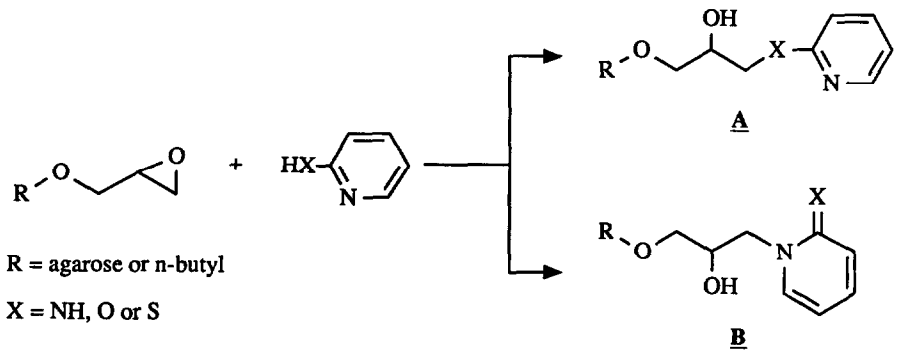
Abstract. The reaction of 2-thio-pyridine N-oxide, 2-amino-, 2-hydroxy, 2-thio-, and 4-thiopyridine with butyl glycidyl ether was investigated as a model system for the functionalization of 2,3-epoxypropyl activated agarose. Unambiguous structural assignment of the products was provided by selective INEPT and nuclear Overhauser difference spectra. All reactions were shown to give only one of the possible regioisomers. Further conclusions regarding the structure of the agarose derivatives were drawn from IR spectra.

Introduction. Agarose functionalized with pyridine derivatives provides a promising new tool for the separation of immunoglobulins and α_2-macroglobulin from serum.[1,2] Since the protein adsorption varies considerably with the agarose substituent, further developments require knowledge of the structures of the agarose derivatives. Theoretically, the SN2 reaction between 2,3-epoxypropyl activated agarose and any of the pyridine derivatives employed in the functionalization reactions may give two different reaction products, as the nucleophilic attack usually occurs on the less substituted epoxide carbon: Scheme 1. To differentiate between these polymeric products by NMR spectroscopy in solution is not possible, and solid state NMR provided no structural information. IR spectra might show the presence of B due to the C=X bond, but in the present cases would not reveal the presence of any minor isomer.



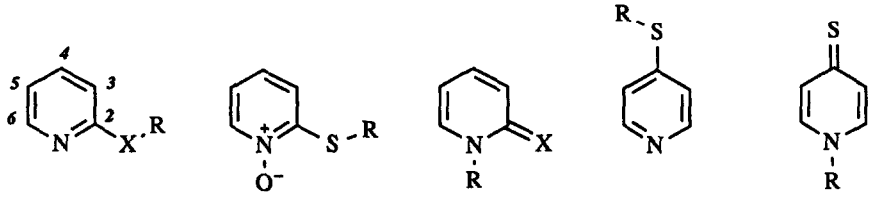
Scheme 1

Therefore, we studied the functionalization reactions with low-molecular weight analogs of the activated agarose, using n-butyl glycidyl ether as starting material under the conditions optimized for glycidyl ether modified agarose.[1]

Results and discussion. Under reaction conditions similar to those optimized for 2,3-epoxypropyl activated agarose,[3] all reactions yielded only one essentially pure product: Scheme 2. A large excess of the pyridine derivative has to be used with the activated agarose to assure reasonable reaction times and was maintained also in the present study. The resulting high concentration of pyridine derivatives in the aqueous phase upon work-up explains the modest yields of products, which partly dissolved in the aqueous phase together with excess starting material. No attempt was made to recover the material from the aqueous phase. However, NMR and TLC showed that no other reaction products were present in the aqueous phase. A reference sample of **1a** was prepared under basic conditions as reported for related compounds.[4]

The ^{1}H NMR spectra reveal the signals of the geminal protons on C-1' as two double doublets and a complex multiplet for H-2'. All other methylene protons appear as complex multiplets, with the signals of H-3' and H-4' overlapping. This assignment is corroborated by coupling information from COSY spectra,[5] but could in part be based upon chemical shift values and signal multiplicity alone. The ring protons in compounds **1** - **3** give the expected pattern of multiplets. If the signal of H-6 is assumed to be that found at lowest field,[6] the assignment of the other ring proton signals follows from the multiplet pattern or coupling information of the COSY spectra (Tables 3 and 4).

The assignment of the ^{13}C NMR signals is derived from the proton assignment via C,H-correlated spectra (HETCOR).[7] Here, the non-protonated ring carbon C-2 shows no correlation, and is further revealed by its lower signal intensity in the one-dimensional spectrum.



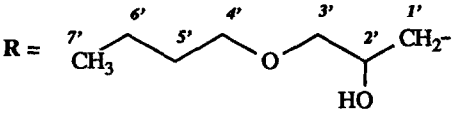
1a, X = NH
1b, X = S

2

3a, X=NH
3b, X=O

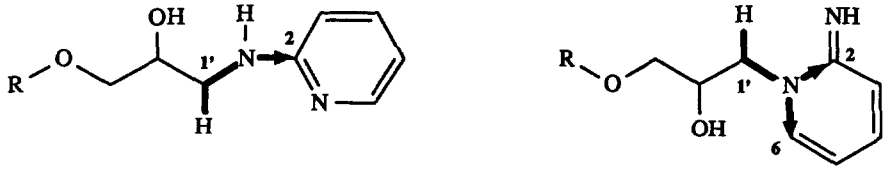
4

5



Scheme 2

Up to this point, no information about the connection between the pyridine ring and the substituent, i.e., the regioselectivity of the reaction, is provided. Resorting to chemical shift arguments, probably by aid of reference compounds, would be a potentially ambiguous approach. For example, as is evident from Table 2, the corresponding carbon atoms in **1a**, **3a** and **3b** exhibit no characteristic chemical shift differences, and in addition, reference compounds are not easily accessible in all cases. An unambiguous structural assignment can be based upon coupling information instead. In particular, proton-carbon magnetization transfer via long-range coupling $^{n}J_{CH}$ (i.e., over two or three bonds) provides valuable connectivity information. This is utilized in the selective INEPT experiment.[8] Here, all proton pulses are applied as selective pulses to only one proton signal, and the resulting carbon spectrum contains exclusively signals of carbons which are long-range coupled to this particular proton. With the selective pulses applied to one H-1', compounds substituted at the exocyclic heteroatom (**A** in Scheme 1) should give a spectrum containing the signal of the unprotonated ring atom C-2 only (additional signals for C-2' and C-3' may be present as well, but are without analytical value in our case). On the other hand, the selective INEPT spectrum of a compound substituted on the ring nitrogen atom (**B** in Scheme 1), should contain an additional signal, i.e. C-6. Thus, a single NMR experiment is sufficient to determine the site of attachment, depending upon whether a spectrum with one or with two ring carbon signals is obtained. This is illustrated in Scheme 3 and Figure 1 for **1a** and **3a**. Only the H-1' signals have to be identified to apply the technique, and spectra are obtained within a few minutes.



1a **3a**

Scheme 3: Magnetization transfer from H-1' to the ring carbons in the selective INEPT experiment (R = n-butyl).

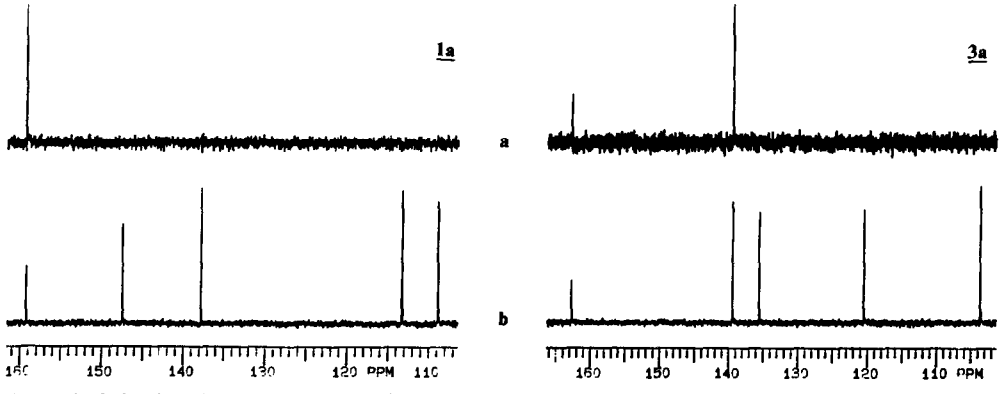


Figure 1: Selective INEPT spectra for **1a** and **3a**, with the selective proton pulses applied to the upfield H-1' signal. a, selective INEPT spectrum; b, reference spectrum.

When the selective pulses are applied to one of the ring protons instead, the full proton and carbon assignment in the ring becomes possible without having to rely on chemical shift arguments alone, as is obvious from Table 4. Signals due to magnetization transfer over three bonds dominate the spectra due to the larger size of the coupling constants, $^3J_{CH}$, as compared with $^2J_{CH}$. The signals for C-3' and C-4' are distinguishable from the selective INEPT spectra for H-1', where only the former carbon shows up.

Since the signal intensity in the selective INEPT spectra depends upon delays in the pulse sequence, which are derived from assumed values for $^nJ_{CH}$,[9] the results were verified by an independent method, i.e., the nuclear Overhauser enhancement[10] between H-1' and the ring protons. For **1a**, **1b** and **2**, the intensity of H-3 should be enhanced upon irradiation of H-1'. For **3a** and **3b**, the intensity of H-6 should increase. This is in accordance with the experimental results: Table 5. For example, **3a** shows a positive NOE for the proton signal at 6.95 ppm (Figure 2). This proton is attached to the same carbon (δ = 139.3 ppm, correlation from the HETCOR spectrum), that appears in the selective INEPT spectrum when the proton pulses are applied to H-1'. Thus, the proton must be H-6 and not H-3 (Scheme 3). In the case of the side-chain substituted compounds **1** and **2**, the identification of the enhanced ring proton H-3 as such is straightforward from the selective INEPT and COSY spectra. For example, in **1b** two ring protons are long-range coupled to the non-protonated carbon C-2. The one at 7.49 ppm is identified as H-4 from its multiplet pattern, and couples to H-3 at 7.28 ppm (COSY spectrum), which is enhanced upon irradiation of H-1' (NOE difference spectrum). The remaining signals are then assigned from the COSY and HETCOR spectra.

Since the choice of the delay lengths in selective INEPT experiments has repeatedly been a matter of discussion,[11] a range of values was tested for compound **3b**. However, rather long delays corresponding to long-range couplings $^nJ_{CH}$ of 6 Hz, i.e. 83 msec, gave the highest signal intensities.

In the selective INEPT spectrum of **4** only the non-protonated ring carbon C-4 is expected to show up, whereas a signal for the protonated carbon C-2 would be expected for the alternative structure **5**. The result (Table 4) points unequivocally to **4**. In this case, the assignment of the ring proton resonances has to be based upon the nuclear Overhauser enhancement, since the selective INEPT spectra run for both ring protons give a similar enhancement of C-4.

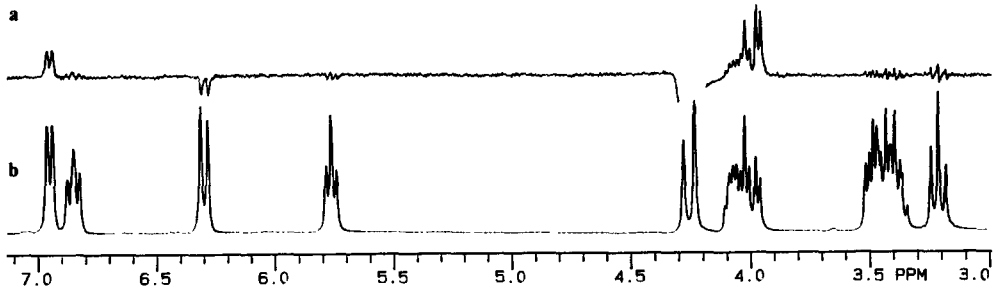


Figure 2: a, NOE difference spectrum for **3a**; b, reference spectrum.

Table 1: ^{1}H NMR chemical shift data of the synthesized pyridine derivatives.

Compound[a]	Chemical shift[b]											
	ring protons				3-butoxy-2-hydroxypropyl protons							
	H-3	H-4	H-5	H-6	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'	H-7'	OH
1a	6.43	7.37	6.55	8.00	3.36, 3.57	3.94	3.44	3.44	1.54	1.34	0.90	3.4[c]
1b	7.28	7.49	6.91	8.23	3.28, 3.40	4.09	3.53	3.49	1.58	1.37	0.92	5.32
2	7.43	7.31	7.11	8.27	3.04, 3.19	4.05	3.57	3.49	1.57	1.37	0.92	4.25
3a · HCl[d]	7.23	7.89	6.98	7.84	4.25, 4.41	4.32	3.62	3.62	1.58	1.38	0.93	-
3a	6.30	6.85	5.76	6.95	3.98, 4.25	4.06	3.21, 3.49	3.41	1.54	1.37	0.92	2.88[e]
3b	6.57	7.38	6.22	7.42	3.91, 4.30	4.12	3.41-3.49		1.55	1.37	0.91	4.70
4	7.13	8.31[f]	7.13	8.31	3.08, 3.18	3.95	3.49	3.43	1.52	1.33	0.89	4.15

[a]For formulae, see Scheme 2. [b]All chemical shifts in ppm relative to TMS, solvent: $CDCl_3$. [c]N-H at 4.91 ppm. [d]Solvent: D_2O, referenced to sodium 3-(trimethylsilyl)-1-propanesulfonate. [e]In DMSO-d_6 at 100°C; N-H at 6.20 ppm. [f]H-2.

Table 2: ^{13}C NMR chemical shift data of the synthesized pyridine derivatives

Compound[a]	Chemical shift[b]											
	ring carbons					3-butoxy-2-hydroxypropyl carbons						
	C-2	C-3	C-4	C-5	C-6	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'	C-7'
1a	158.9	108.7	137.5	113.1	147.2	46.1	70.5	72.5	71.3	31.7	19.3	13.9
1b	158.9	122.4	136.2	119.6	148.4	35.1	70.1	73.1	70.9	31.4	19.0	13.6
2	152.3	122.1	126.6	120.4	138.6	34.1	68.6	72.9	71.2	31.4	19.1	13.7
3a ·HCl[c]	157.2	117.8	145.5	116.3	143.0	58.6	69.3	74.1	73.6	33.3	21.2	15.8
3a	162.7	120.4	135.5	103.5	139.3	57.7	71.0	71.9	71.5	32.1	19.7	14.2
3b	163.5	119.9	139.9	106.0	139.1	53.3	68.6	71.6	70.9	31.2	18.9	13.5
4	148.7	120.6	149.1	120.6	148.7	34.2	68.6	73.0	71.2	31.5	19.1	13.7

[a]For formulae, see Scheme 2. [b]Chemical shifts in ppm relative TMS, solvent: $CDCl_3$ unless indicated otherwise. [c]Solvent: D_2O, referenced to sodium 3-(trimethylsilyl)-1-propanesulfonate.

Table 3: Proton coupling constants

Compound[a]	H-3	H-4	H-5	H-6	H-1'$_{downfield}$	H-1'$_{upfield}$
1a	8.7	7.0, 8.7	5.0, 7.0	5.0	2.9, 6.1, 13.7[b]	5.0, 6.5, 13.7[b]
1b	8.0	7.2, 8.0	5.0, 7.2	5.0	5.0, 14.5	6.4, 14.5
2	8.2	7.5, 8.2	6.5, 7.5	6.5	5.3, 13.5	5.3, 13.5
3a · HCl[d]	8.6	7.0, 8.6	6.8, 7.0	6.8	2.9, 13.5	8.0, 13.5
3a	9.0	6.4, 9.0	6.4, 6.8	6.8	1.3, 13.5	5.5, 13.5
3b	9.0	6.6, 9.0	6.6, 6.6	6.6	2.6, 13.5	6.4, 13.5
4	6.5	6.5[c]	6.5	6.5	5.3, 14.0	7.4, 14.0

[a]For formulae, see Scheme 2. [b]Couples to N-H. [c]H-2.

Table 4: Ring proton and carbon assignment of the reaction products by HETCOR, COSY, and selective INEPT spectra.

Compound	^{13}C chemical shift (ppm)[a]	^{1}H chemical shift (ppm)[a]			Assignment[c]
		HETCOR	COSY	selective INEPT[b]	
1a	158.9	-		3.57, 8.00, 7.37	**C-2**
	108.7	6.43	7.37	6.55	C-3
	137.5	7.37	6.43, 6.55	8.00, 6.55	C-4
	113.1	6.55	7.37, 8.00	6.43, 8.00	C-5
	147.2	8.00	6.55	7.37, 6.55	C-6
1b	158.9	-		3.28, 7.49, 8.23	**C-2**
	122.4	7.28	7.49	6.91, 7.49	C-3
	136.2	7.49	7.28, 6.91	8.23	C-4
	119.6	6.91	7.49, 8.23	7.28, 8.23	C-5
	148.4	8.23	6.91	6.91, 7.49	C-6
2	152.3	-		3.04, 3.19, 7.31, 8.27	**C-2**
	122.1	7.43	7.31	7.11	C-3
	126.6	7.31	7.11, 7.43	8.27	C-4
	120.4	7.11	7.31, 8.27	7.43, 8.27	C-5
	138.6	8.27	7.11	7.11, 7.31	C-6
3a · HCl	157.2	-		4.41, 7.23	**C-2**
	117.8	7.23	7.89	6.98	C-3
	145.5	7.89	6.98, 7.23		C-4
	116.3	6.98	7.84, 7.89	7.23	C-5
	143.0	7.84	6.98	4.41, 6.98	**C-6**
3a	162.7	-		4.25, 6.30, 6.85, 6.95	**C-2**
	120.4	6.30	6.85	5.76	C-3
	135.5	6.85	5.76, 6.30	6.95	C-4
	103.5	5.76	6.85, 6.95	6.30, 6.95	C-5
	139.3	6.95	5.76	4.25, 5.76, 6.85	**C-6**
3b	163.5	-		3.91, 4.30, 7.38, 7.42	**C-2**
	119.9	6.57	7.38	6.22	C-3
	139.9	7.38	6.22, 6.57	7.42	C-4
	106.0	6.22	7.38, 7.42	6.57, 7.42	C-5
	139.1	7.42	6.22	3.91, 4.30, 6.22, 7.38	**C-6**
4	148.7	8.31	n.d.	7.13, 8.31	C-2
	120.6	7.13		7.13, 8.31	C-3
	149.1	-		3.08, 3.18, 8.31	**C-4**
	120.6	7.13		7.13, 8.31	C-5
	148.7	8.31		7.13, 8.31	C-6

[a] Chemical shift reference and solvents: Tables 1 and 2. [b] Selective INEPT experiment; the denoted carbon signal was observed when the selective pulses were applied to one of the listed proton resonances. [c] Bold face identifies those signals which appeared in the selective INEPT experiment with selective proton pulses applied to one H-1' signal.

Table 5: Nuclear Overhauser effects from NOE difference spectra

Compound	NOE observed upon irradiation of upfield H-1'
1a[a]	H-3 (12%), H-6 (3%), H-2' (4%)
1b	H-3 (1%), H-2' (5%)
2	H-3 (7%), H-1'(19%)[b]
3a · HCl[a]	H-6 (6%), H-5 (-1%)
3a[a]	H-6 (8%), H-3 (-2%),[c] H-1' (≈20%)[b]
3b	H-6 (8%), H-5 (-1%), H-1' (22%)[b]
4	H-3 (6%), H-2 (-1%), H-1' (16%)[b]

[a]Downfield H-1' signal irradiated. [b]Downfield H-1' signal enhanced.[c]Indirect effect via N-H.

The IR spectra of 1, 2 and 4 allow no conclusion about the type of product formed. A reportedly strong C=S stretching band, which should occur in the region between 1142-1112 cm^{-1} (ortho position) or between 1119-1108 cm^{-1} for compounds of the type 3 (X = S) and 5,[12] would be obscured by the strong C-O stretching bands of the alcohol. In the IR spectrum of 3b, a band at 1660 cm^{-1} indicates the pyridone carbonyl group, i.e. nitrogen substitution.[13] For 3a, the C=NH group is indicated by a strong band at 1670 cm^{-1}.

Agarose functionalized with 2-hydroxypyridine shows a strong C=O stretching band around 1660 cm^{-1} corresponding to the one described for 3b. A C=NH stretching band at 1670 cm^{-1} is present in the IR spectrum of the agarose derivatized with 2-aminopyridine, thus resembling the spectrum of 3a.[14]

On the other hand, the IR spectra of the thiopyridine functionalized agaroses allow no conclusion regarding the product isomer, since also in this case a C=S stretching band would be obscured by the C-O stretching bands. The structural assignment in this case must rest solely on analogy with the products described in this work. However, because of the high regioselectivity of the reactions between thiopyridines and butyl glycidyl ether, it is reasonable to conclude that the corresponding type of product will be formed from the activated agarose as well.

Conclusions. Selective INEPT spectra provide a convenient and reliable way to assign the structure of the pyridine derivatives described in this study. The assignment can be corroborated by the homonuclear Overhauser effect between ring and substituent protons. It was shown that the reactions between butyl glycidyl ether and pyridine derivatives selectively yield only one of the two possible isomers in all cases. Due to this high selectivity, it is concluded that the corresponding isomers are formed when 2,3-epoxypropyl activated agarose is reacted under identical conditions. This is supported by IR spectra in case of the 2-aminopyridine and 2-hydroxypyridine derivatives.

Experimental

^1H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, on a Varian XL 300 spectrometer using manufacturer-supplied software. The sample amount was about 50 mg in 0.7 ml solvent. For the ^{13}C NMR spectra, a 1 s delay between pulses and 0.9 s acquisition time were used. COSY-45 spectra[5] were acquired with a spectral width of 2400 Hz in both dimensions. 1K data points were acquired for each of 256 increments with 4 transients and 2 dummy scans per increment, and a relaxation delay of 1 s, giving a total experiment time of 0.5 h. The data were zero filled to 1K in f_1 and pseudo-echo-weighted prior to the Fourier-transformation. HETCOR spectra[7] were run over a spectral width of 11400x2400 Hz, 128 increments with 8 transients and 1 dummy scan each were recorded, using a delay of 1 s. The total experimental time was 21 min. Zero filling in f_1 gave a 2K x 0.5K data matrix, which was pseudo-echo-weighted and Fourier-transformed. The selective INEPT spectra employed selective proton pulses (width: 25 Hz, duration: 20 ms) and were recorded with the delay parameters adjusted for C-H long-range coupling constants of 6 Hz ($\Delta_1 = \Delta_2 = 83$ ms).[8] 128 transients with a recycling time of 2.2 s were obtained, giving a total time of 5 min for each experiment. NOE difference spectra were recorded on non-degassed 10 mg samples in D_2O, or on degassed samples (freeze-pump-thaw cycle) in $CDCl_3$, with the decoupler on during a delay of 5 s, and off during acquisition. A reference spectrum, where an empty spectral region was irradiated was subtracted from these spectra after Fourier-transformation.[10] 160 transients were obtained, giving a total of 23 min per irradiated proton signal. ^{13}C NMR spectra of crude products were recorded at 15 MHz (Jeol JNM-FX60 spectrometer). Infrared spectra were obtained on a Perkin-Elmer model 177 spectrometer. The mass spectra were obtained at 70 eV on a Finnigan MAT INCOS 50 spectrometer. Elemental analyses were performed by Mikro Kemi AB, Uppsala.

All reactions, except the synthesis of **1a**, were completed by stirring the reaction mixture for 3 h at ambient temperature and under a nitrogen atmosphere (not for **3a** and **3b**). Crude products were isolated by extracting the reaction mixture with an equal amount of diethyl ether and evaporation of the solvent after drying the solution over sodium sulfate. Flash chromatography was performed on silica (0.032 - 0.060 mesh).

Syntheses

2-(3'-Butoxy-2'-hydroxypropylamino)pyridine (1a). 2-Aminopyridine (2.5 g, 27 mmol) in dry benzene (6 ml) was treated with 50% sodium amide in toluene (2.5 g, 32 mmol) and, after addition of butyl glycidyl ether (2.5 g, 19 mmol) the mixture was refluxed overnight. Brine (100 ml) was added and the mixture extracted with diethyl ether (3x100 ml). The combined organic phases were washed with brine (3x100 ml) and dried over sodium sulfate, giving 4.24 g of a brown oil. Flash chromatography over silica (ethyl acetate) gave 2.8 g (12.5 mmol, 66%) **1a** as an orange oil (pure by ^1H NMR), which crystallized from n-hexane/isopropanol in colourless plates, m.p. 54-55 ^0C. ^1H NMR: Table 1; ^{13}C NMR: Table 2; IR (KBr): 3313, 3103, 2956, 2930, 2870, 1624, 1537, 1464, 1421, 1294, 1156, 1141, 1105, 769, 739 cm^{-1}; MS, m/z (rel. intensity) = 224(2, M^+), 137(39), 119(6), 108(26), 107(100), 94(11), 79(15), 78(28). Anal.

calcd. for $C_{12}H_{20}N_2O_2$: C, 64.3, H, 8.9, N, 12.5, O, 14.3; found: C, 64.4, H, 9.0, N, 12.4, O, 14.3.

2-(3'-Butoxy-2'-hydroxypropylthio)pyridine (1b). 2-Mercaptopyridine (1.00 g, 9 mmol) was dissolved in 0.1 M sodium phosphate buffer (pH 7.0, 20 ml), sodium borohydride (0.5 g) was added and the pH of the mixture adjusted to 6.8 with 2 M HCl. This solution was added to butylglycidyl ether (0.26 g, 2 mmol) in a 25 ml round-bottom flask. The crude product, 0.71 g, was purified by flash chromatography (diethyl ether/chloroform/conc. NH_3 (aqueous) 9:1:0.1), yielding 0.43 g (1.8 mmol, 89%) 1b as a yellow oil. ^1H NMR: Table 1; ^{13}C NMR: Table 2; IR(neat): 3400, 2940, 2860, 1580, 1550, 1450, 1415, 1125, 750 cm^{-1}; MS, m/z (rel. intensity) = 240(1, $[M-1]^+$), 154(100), 124(21), 125(18), 111(42), 78(31). Anal. calcd. for $C_{12}H_{19}NO_2S$: C, 59.8, H, 7.9, N, 5.8, O, 13.3, S, 13.3; found: C, 59.2, H, 8.1, N, 5.8, O, 14.2, S, 13.3.

2-(3'-Butoxy-2'-hydroxypropylthio)pyridine-N-oxide (2). 2-Mercaptopyridine N-oxide (1.40 g, 11 mmol) was dissolved in 0.1 M sodium phosphate buffer (pH 7.0, 20 ml), sodium borohydride (0.5 g) was added and the pH of the solution was adjusted to 6.8 with 2M HCl. This solution was added to butyl glycidyl ether (0.26 g, 2 mmol) in a 100 ml round-bottom flask. The crude product, 0.54 g, was purified by flash chromatography (ether/methanol/conc. aqueous NH_3 9:1:0.1) yielding 0.30 g (1.2 mmol, 58%) 2 as an oil. ^1H NMR: Table 1; ^{13}C NMR: Table 2; IR (neat): 2940, 2860, 1590, 1550, 1470, 1420, 1270, 1220, 1120, 830, 750, 700 cm^{-1}; MS, m/z (rel. intensity) = 258(4, $[M+1]^+$), 240(1), 222(6), 170(53), 154(21), 127(100), 78(59). Anal. calcd. for $C_{12}H_{19}NO_3S$: C, 56.0, H, 7.4, N, 5.4, O, 18.7, S, 12.4; found: C, 55.2, H, 7.4, N, 5.5, O, 18.9, S, 12.0.

1-(3'-Butoxy-2'-hydroxypropyl)-2-pyridone-imine (3a). 2-Aminopyridine (3.30 g, 35 mmol) was dissolved in 0.1M sodium phosphate buffer (pH 7.0, 40 ml) and the pH of the mixture was adjusted to 6.8 with 2M HCl. This solution was then added to butyl glycidyl ether (0.26 g, 2 mmol) in a 100 ml round-bottom flask. The crude product, 0.5 g, was purified by flash chromatography (methanol/isopropanol 9:1, followed by methanol/conc. HCl 10:0.1) yielding 0.26 g (1.0 mmol, 50%) 3a-hydrochloride as colourless crystals. Conversion to the free base was effected by adjusting the pH of a solution of the hydrochloride in water with 2 N NaOH to pH 12 and extracting the resulting emulsion with diethyl ether. After drying (sodium sulfate) and evaporation of the solvent, 3a remained as a brown oil. 3a-Hydrochloride: ^1H NMR: Table 1; ^{13}C NMR: Table 2; IR (neat): 3700-3000, 2960, 2880, 2500, 2300, 1670, 1590, 1520, 1460, 1405, 1255, 1170, 760 cm^{-1}; MS, m/z (relat. intensity) = 224(3, M^+), 167(6), 137(17), 108(10), 94(14), 38(33), 36(100). Anal. calcd. for $C_{12}H_{21}ClN_2O_2$: C, 55.3, H, 8.1, Cl, 13.6, N, 10.8, O, 12.3; found: C,54.4, H, 8.0, N, 11.7, O, 11.7. 3a: ^1H NMR: Table 1; ^{13}C NMR: Table 2; IR (neat): 3750-3076, 3315, 2932, 2868, 1651, 1645, 1568, 1538, 1463, 1105, 750 cm^{-1}; MS, m/z (rel. intensity) = 224(9, M^+), 167(36), 137(92), 119(20), 108(69), 107(22), 95(57), 94(100), 78(30). Anal. calcd. for $C_{12}H_{20}N_2O_2$: C, 64.3, H, 8.9, N, 12.5, O, 14.3; found: C, 63.4, H, 9.0, N, 12.0, O, 14.9.

1-(3'-Butoxy-2'-hydroxypropyl)-2-pyridone (3b). 2-Hydroxypyridine (3.30 g, 34.4 mmol) was dissolved in 1M NaOH (20 ml). This solution was added to butyl glycidyl ether (0.26 g, 2 mmol) in a 50 ml round-bottom flask. The crude product, 0.99 g, was dissolved in diethyl ether/conc. acetic acid 10:0.1 and purified by flash chromatography (diethyl ether/conc. acetic acid 10:0.1, followed by diethyl ether), yielding 0.16 g (0.7 mmol, 36%) 3b as an oil. ^1H NMR: Table 1; ^{13}C NMR: Table 2; IR (neat): 2940, 2860, 1660, 1605, 1570, 1540, 1460, 1430, 1245, 1150, 1100, 990, 760, 720; MS: m/z (rel. intensity) = 225(2, M^+), 207(18), 150(26), 138(100), 109(39), 96(88), 80(34). Anal. calcd. for $C_{12}H_{19}NO_3$: C, 64.0,

H, 8.4, N, 6.2, O, 21.3. Found: C, 63.3, H, 8.5, N, 6.4, O, 22.0.

4-(3'-butoxy-2'hydroxypropylthio)pyridine (4). 4,4'-Dipyridyl disulfide (0.50 g, 2.3 mmol) was dissolved in 0.1M sodium phosphate buffer (pH 7.0, 20 ml). The pH of the mixture was adjusted to 6.8 with 2M HCl. This solution was added to butyl glycidyl ether (0.26 g, 2 mmol) in a 100 ml round-bottom flask. The crude product, 0.63 g, was purified by flash chromatography (diethyl ether) yielding 0.20 g (0.83 mmol, 42%) 4 as a yellow oil. ^{1}H NMR: Table 1; ^{13}C NMR: Table 2; IR (neat): 2940, 2860, 1580, 1530, 1480, 1410, 1220, 1110, 1060, 1000, 800, 705; MS, m/z (relat. intensity) = 241(2, M^+), 223(2), 125(17), 113(35), 57(100). Anal. calcd. for $C_{12}H_{19}NO_2S$: C, 59.8, H, 7.9, N, 5.8, O, 13.3, S, 13.3; found: C, 59.2, H, 8.2, N, 5.7, O, 14.3, S, 12.6.

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