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Negatively charged yellow-emitting 1-aminopyrene dyes for reductive amination and fluorescence detection of glycans

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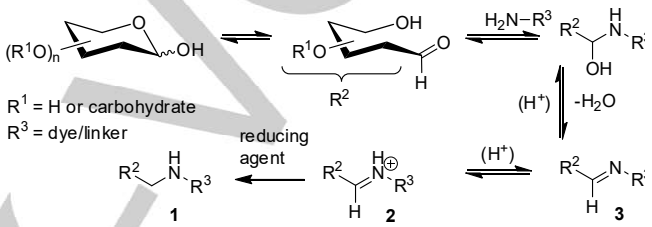
Abstract: *1-Aminopyrenes with three ω-hydroxylated N-alkylsulfonamido or alkylsulfonyl residues in positions 3, 6 and 8 were prepared, O-phosphorylated and applied for reductive amination of oligosaccharides. The dyes (ε ~ 20000 M^{-1} cm^{-1}) with six negative charges (pH ≥ 8) and low m/z ratios enable labeling and fluorescence detection of reducing sugars (glycans) related to the most structurally and functionally diverse class of natural products. Under excitation with a 488 nm laser, the new glycoconjugates emit yellow light of about 560 nm, outperforming (in respect of brightness and faster electrophoretic mobilities) the corresponding APTS derivatives (benchmark dye with green emission in conjugates).*

Glycosylation is an enzymatically driven and highly diverse transformation of proteins, lipids or other noncarbohydrates. The products of glycosylation (glycoconjugates) have a new chemical bond formed between a carbohydrate (glycan; donor) and other molecule (acceptor). Glycoconjugates represent one of the most structurally and functionally diverse class of natural products involved in fundamental biochemical processes in living matter.[1] Only few specific functions of these complex and carbohydrate-rich molecules have been well understood so far.[2] Further progress in glycomics and glycobiology depends on the advances in analytic techniques applicable to complex carbohydrates. Carbohydrates do not absorb visible light, and for the sensitive detection by emission they need to be labeled with a fluorescent tag.[3] Capillary gel electrophoresis (CGE) is an important method for analyzing glycoconjugates including glycoproteins, glycopeptides and "released" (enzymatically cleaved from the acceptors) *N*- or *O*-glycans.[4] The net electrical charge is required for separation of the analytes by CGE. The native carbohydrates, except sialic or glucuronic acids, sulphated or phosphorylated derivatives, are electroneutral and cannot be separated by their mass to charge ratio (electrophoresis). Importantly, the features of an "ideal" fluorescent label required for CGE – the electrical charge, emissive properties and the reactive group – can be incorporated in one fluorescence dye with (multiple) charges and the amino group reacting with aldehyde residues in reducing sugars (Scheme 1). Combined with laser induced fluorescence detection (LIF), this method allows fast and very fine resolution of analytes according to their mass to charge ratio (*m/z*) and hydrodynamic radius.[5] A high throughput analysis of fluorescent glycan derivatives is performed on commercial DNA sequencers equipped with a CGE-LIF module.[5c] The fluorescent label (R^3-NH_2 in Scheme 1) applicable in CGE-LIF must have an amino group suitable for reductive amination, high electrophoretic mobility and "brightness" (which is the product of the fluorescence quantum yield and absorption coefficient at the excitation wavelength).



Scheme 1. Reductive amination of mono and oligosaccharides.

APTS (Scheme 2) emerged as a unique dye for reductive amination and detection of glycans.[5,6,7] The fluorescence of APTS derivatives is captured in the "green" color channel of the standard DNA sequencers.[5c] Conjugates of APTS are excitable with an argon laser (emission lines 488 nm and 514 nm).[5c,5e] However, the performance of APTS as a fluorescent tag providing only one emission color, moderate brightness and three negative charges, is limited. To facilitate further progress in glycomics, glycobiology and analytical chemistry of complex carbohydrates, we developed bright fluorescent dyes with an aromatic amino group, multiple negative charges and yellow emission.

Design and synthesis of the new pyrene dyes. The high-performing fluorescent tags applicable in the reductive amination and CGE-LIF of glycans must have an amino group with pK_a *of the conjugated acid* in the range of 3–4 for the efficient reaction (Scheme 1) *at pH ~ 3*, net charge of –3...–6 *at pH = 8* (pH of the buffer solution in CE) to provide high electrophoretic mobility, solubility in aqueous buffers and stability against reduction with boranes or borohydrides in a wide pH range (3–8). The absorption at 488 nm (ε_{488}) or 505 nm (ε_{505}) determined the excitation efficiency with an argon ion laser or solid state laser; high brightness and the minimal cross-talk with the "APTS channel" in the detector (low emission of conjugates at 520 nm) are also required. These features are set by the reaction conditions in Scheme 1, and the properties of the standard DNA sequencing equipment used for the separation and detection of the fluorescent glycan derivatives. APTS is a reference dye with green emission in conjugates with oligosaccharides (see Figure 2 and Supporting Information). We designed new dyes with yellow emission in conjugates, trying to minimize the interference with an APTS detection window.

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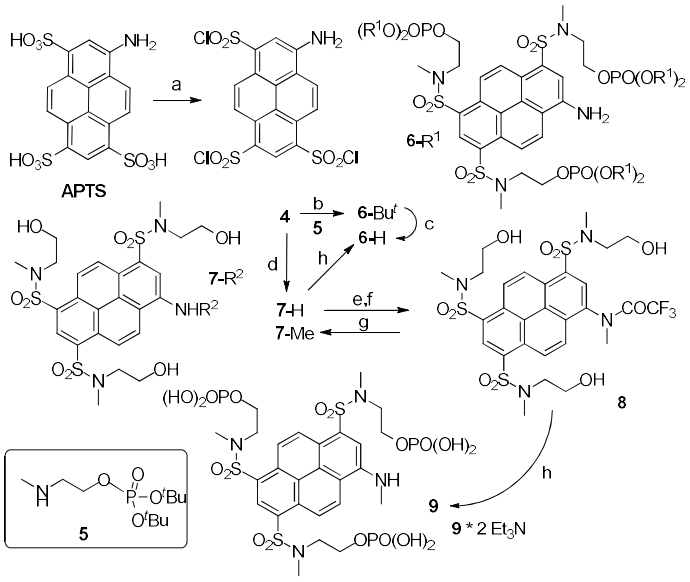

Scheme 2. Triple *O*-phosphorylated 1-aminopyrene trisulfonamides: a) $ClSO_3H$, 65°C, 3 h; b) **5**, Et_3N, MeCN, r. t., overnight; c) CF_3CO_2H, r. t., 1 h, then 1 M aq. $Et_3N{*}H_2CO_3$ (pH 8–9), overnight; d) $CH_3NHCH_2CH_2OH$, aq. MeCN, r. t., overnight; e) $(CF_3CO)_2O$, CH_2Cl_2, Et_3N, then CH_3I, Cs_2CO_3, DMF, 70 °C, 40 min; f) MeOH, $NaHCO_3$; g) aq. NaOH, MeOH, r. t.; h) $POCl_3$, $(MeO)_3PO$, r. t., 3 h, then aq. $Et_3N{*}H_2CO_3$ buffer (pH 8–9), overnight.

Table 1. Spectral properties of the dyes and their *m/z* ratios

Dye *(m/z)*	Absorption λ_{max}, nm (ε, M^{-1} cm^{-1})	Emission λ_{max}, nm (Φ_{fl})[a]	*Solvent*	Fluor. lifetime τ, ns
APTS[b] *(151)*	424 (20600)	500 (0.95)	aq. PBS	-
APTS-G6[c]	455 (17000)	511	H_2O	-
6-H (144)	471	544 (0.88)	H_2O	5.9
7-H	477 (22400)	535 (0.96)	MeOH	5.6
7-Me	493 (23000)	549 (0.97)	MeOH	5.9
9	502	563 (0.85)	H_2O	3.6
13	502 (23400) 509 (19500)	550 (0.88) 563 (0.67)	MeOH H_2O	6.3 6.4
15	486 (21000)	534 (0.80)[d,e]	MeOH	4.9
16 *(137)*	477 (19600)	542 (0.92)	TEAB[f,g]	5.8

[a] absolute values of the fluorescence quantum yields (if not stated otherwise); [b] data from ref. [6b], abs. measured in H_2O, emission - in aq. phosphate buffer at pH =7.4; [c] conjugate with maltohexaose (G_6), data from ref. [5b]; [e] excitation at 375 nm; [e] Rhodamine 6G as a ref. dye with Φ_{fl} = 0.9; [f] aq. $Et_3N{*}H_2CO_3$, pH = 8–8.5; [g] fluorescein as a ref. dye with Φ_{fl} = 0.9 in 0.1 M NaOH.

For achieving this goal, we converted the sulfonic acid residues in APTS (Scheme 2) into more powerful electron acceptors – sulfonamides. Sulfonamides (represented by dyes **6**–**9** in Scheme 2) have higher values of the *Hammett* σ-constants (σ_m = 0.53, σ_p = 0.60 for SO_2NH_2)[8] than ionized sulfonic acid residues in APTS (σ_m = 0.05, σ_p = 0.09).[9] The presence of an electron-donating (*N*-alkyl)amino group and the acceptor groups in "active" positions (*3*, *6* and *8*) of the pyrene system leads to the "push-pull" dyes[10] emitting blue-green (APTS), green (**6**-R^1, **7**-H) or yellow (**7**-Me, **9**) light. The spectral properties of the dyes are given in Table 1 (see also Supporting Information).

To provide multiple negative charges and high electrophoretic mobility at pH 8, primary phosphates (R-OPO_3H_2) are preferred over phosphonates,[11a] because their first and second *pK*a values are in the range of 1.5-1.9 and 6.3-6.8, respectively.[11b] In the electrophoresis buffer solution, one primary phosphate group introduces two negative charges (phophonates are less acidic and not fully ionized at pH 8). APTS[6b] (Scheme 2) was converted into the relatively stable 1,3,6-tris(chlorosulfonyl)pyrene-8-amine (**4**) and then to sulfonamides (**6**-*t*Bu, **7**-H) by reaction with the corresponding amine (**5** or $CH_3NHCH_2CH_2OH$).

Direct phosphorylation of three hydroxyl groups in **7**-H or **8** (reagent *h* in Scheme 2) followed by hydrolysis[12] afforded dyes **6**-H (16%) or **9** bearing six negative charges at pH 8. To improve the yield, we prepared *O*-phosphorylated *N*-(methylamino)ethanol **5**, let it react with compound **4**, isolated the intermediate **6**-*t*Bu and then converted it into dye **6**-H (83%).

Trying to increase the polarity of aminopyrene chromophore even further, we introduced alkyl sulfonyl groups into positions *3*, *6* and *8* of 1-aminopyrene and prepared dyes **13**, **15**, and **16** (Scheme 3). Alkyl sulfonyl substituents have higher values of the *Hammett* σ-constants (σ_m = 0.56-0.66, σ_p = 0.68-0.77 for SO_2Alkyl)[8] than sulfonamides (σ_m = 0.53, σ_p = 0.60 for SO_2NH_2).[8] This indicates that they are more powerful acceptors than sulfonamides (compounds **6**-R^1, **7**-R^2 and **9** in Scheme 2).

We found that trifluoroacetyl residue is a better protecting group for 1-aminopyrene than acetyl,[13] because it is acid-stable, but can be easily cleaved under mild basic conditions. Bromination of 1-(trifluoroacetylamino)pyrene led to tribromide **10** (Scheme 3), an important precursor to the functionally substituted aminopyrenes. Palladium-catalyzed cross-coupling[14] of tribromide **10** with 3-mercapto-1-propanol afforded triol **11** with three alkyl aryl sulfide residues. Oxidation of **11** with hydrogen peroxide in acetic acid in the presence of sodium tungstate[15] led to trisulfonyl derivative **12**. Deprotection of the amino group in compound **12** by heating with aq. NaOH in methanol gave amine **15** (model compound). Another model dye with *N*-methyl group (**13**) was prepared from intermediate **11** by *N*-methylation of trifluoroacetylamino group and mild hydrolysis of the amide group. Phosphorylation of **12** followed by hydrolysis led to aminopyrene **16** with three primary phosphate groups attached to alkyl sulfonyl residues.The phosphate groups in dyes **6**-H, **9** and **16** are hydrolytically stable within a broad range of pH: from 3 (reductive amination conditions) to 8.3 (electrophoresis) and beyond.

The model pyrenes with *N*-methylamino groups (**7**-Me, **9** in Scheme 2 and **13** in Scheme 3) are structurally similar to the final products formed in Scheme 1 upon reductive amination of carbohydrates; they allow to measure the red-shifts in the

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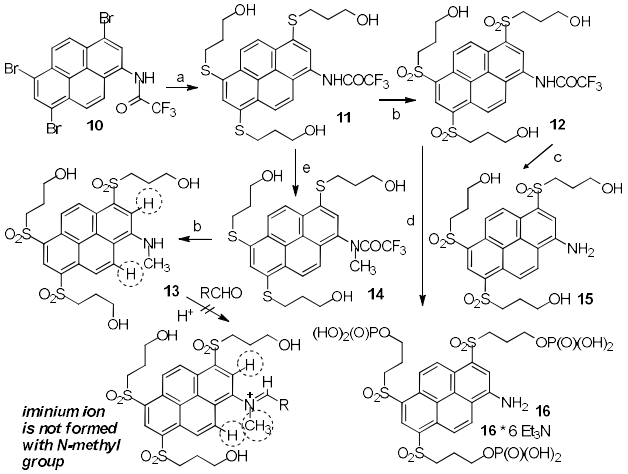

Scheme 3. Triple *O*-phosphorylated 1,3,6-tris[(3-hydroxypropyl)sulfonyl]-pyrene-8-amine **16**: a) $HS(CH_2)_3OH$, DIPEA, $Pd_2(dba)_3$, XantPhos, DMF, 100 °C, overnight; b) aq. H_2O_2, Na_2WO_4, AcOH, r. t., overnight; c) aq. NaOH, MeOH; d) $POCl_3$, $(MeO)_3PO$, r. t., then aq. $Et_3N^*H_2CO_3$ buffer (pH 8–9); e) CH_3I, Cs_2CO_3, DMF.

absorption and emission spectra and extinction coefficients (**7**-Me, **13** in Table 1). Importantly, dyes **7**-Me, **9** and **13** did not participate in the reductive amination of glucose (even under harsh conditions). This result may be explained by the additional steric hindrance pertinent to the planar iminium ion (Scheme 3), in which *two* aromatic hydrogen atoms adjacent to the reaction center are expected "to repulse" the *N*-methyl group.

Spectral properties of dyes and conjugates. The photophysical properties of APTS, its conjugate with maltohexaose (APTS-G_6) and the new pyrenes, are given in Table 1. The dyes in Table 1 form two groups: compounds with a primary amino group (APTS, **6**-H, **7**-H, **15** and **16**) and dyes with a secondary amino group (APTS-G_6, **7**-Me, **9**, and **13**). Pyrenes of the first group absorb at 424 nm (APTS) to 486 nm (**15**). Compounds of the second group relate to the products formed in the course of reductive amination (Scheme 1); their absorption maxima are red-shifted and found in the range from 455 nm (APTS-G_6) to 509 nm (**13**) (in aqueous solutions). For example, *N*-methylation (**6**-H → **9**) shifted the absorption maximum to the red by 31 nm, while the emission underwent bathofluoric shift of "only" 19 nm. Thus, the Stokes shift reduced from 73 nm (**6**-H) to 61 nm (**9**). Importantly, within each group the spectrum of APTS or APTS-G_6 conjugate is separated from the other spectra *of the same group* by 42–69 nm (absorption maxima) and by 38–54 nm (emission maxima): new dyes absorb and emit at longer wavelengths. This feature is important, as the glycan conjugates of dyes **6**-H and **16** are intended to have minimal emission in the APTS detection window. Comparing sulfonamides (**6**-H, **7**-Me) with structurally related alkyl sulfones (**13**, **16**) we observed the red shift of only 6–9 nm in absorption, while the positions and shapes of emission bands remained the same (Table 1 and

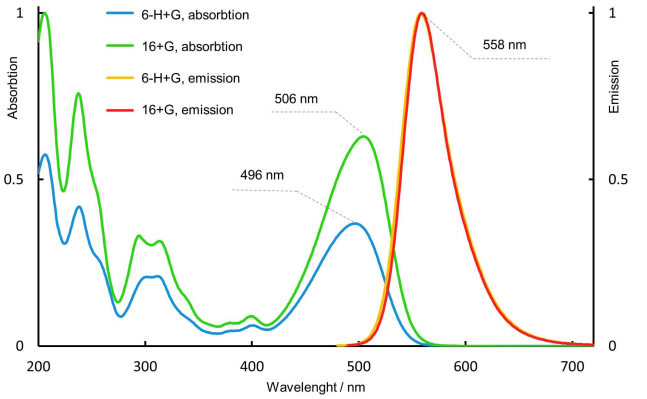

Figure 1. Absorption and emission spectra (aq. $Et_3N^*H_2CO_3$ buffer) of conjugates with glucose prepared from dyes **6**-H and **16**.

Figure 1). The electron-donating capacity of the amino group as a single donor is limited, and this can explain only the small red-shift observed by transition from sulfonamides to alkyl sulfones.
The brightness of a glycan label is termed as a product of the extinction coefficient (at 488 nm, as an excitation wavelength) and the fluorescence quantum yield. The fluorescence quantum yields of dyes **6**-H and **16** (Table 1) are 0.88 and 0.92, respectively . For APTS conjugates, the extinction coefficient at the maximum (455 nm) is 17000 M^{-1} cm^{-1}, and the absorption at 488 nm is ca. 35% of the maximal value.[5b] For all new *N*-alkylated pyrenes (**7**-Me, **9**, **13**, **6**-H+G, **16**+G) we can assume the extinction coefficient at 488 nm to be about 18000 M^{-1} cm^{-1}. Therefore, the conjugates of the new dyes are ca. 3 times brighter than APTS derivatives (under excitation with the 488 nm laser).

Gel electrophoresis. We used APTS as a reference dye, compounds **6**-H and **16** as new reagents, and obtained their conjugates with glucose (G) and oligomers (maltotriose G_3 and maltoheptaose G_7), mannose (M) and oligomers [2-*O*-, 3-*O*- and 4-*O*-(α-D-mannopyranosyl)-D-mannoses (M_2-2O, M_2-3O and M_2-4O), mannotriose (M_3), mannotetraose (M_4)], as well as 3'- and 6'-sialyllactoses. Due to the presence of the strong acceptors – sulfonamido or alkylsulfonyl groups – dyes **6**-H and **16** undergo reductive alkylation more reluctantly than APTS. The detailed procedures for reductive amination[16] and yields of the individual conjugates[17] are given in Supporting Information. Figure 1 shows absorption and emission spectra of glucose conjugates prepared from dyes **6**-H and **16**. The absorption spectra are very similar, and the emission spectra are practically identical. Therefore, we preferentially used dye **16** in reductive amination. Figure 2 shows the gel electrophoresis results obtained with APTS and its conjugates (lanes 1, 3, 5), as well as compound **16** and its conjugates with reducing sugars (lanes 2, 4, 6). The conjugates of dye **16** move faster than the corresponding conjugates of APTS. Due to higher net charge of dyes **6**-H, **16** and their conjugates, the distances between yellow bands related to them are shorter than the distances between green bands of APTS conjugates (Figures 2, S5 and S6). In case of mannobiose isomers, the selectivity profile of dye **16** is different from that of APTS (Figure 2). Conjugates of M_2-2O and M_2-3O with APTS move as one spot (lane 3, fourth spot from the top), and APTS+M_2-4O moves slower

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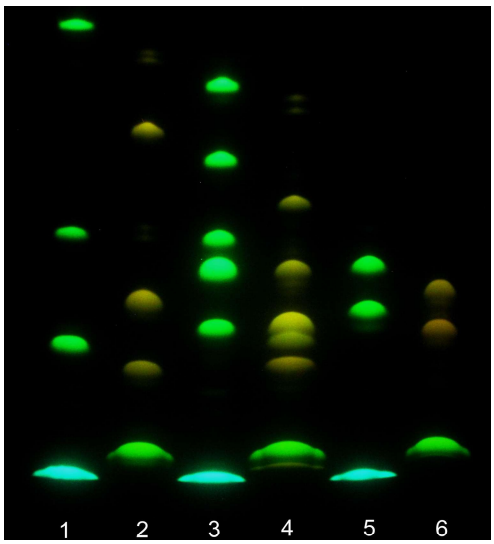

Figure 2. Gel electrophoresis (migration from "north" to "south", pH 8.3); detection by emission (excitation at 365 nm). From down to top. Lane 1: APTS (lowest; blue), APTS+G, APTS+G_3, APTS+G_7 (green). Lane 2: dye **16** (green), **16**+G, **16**+G_3, **16**+G_7 (yellow). Lane 3: APTS, APTS+M, APTS+M_2-2O/APTS+M_2-3O (unresolved), APTS+M_2-4O, APTS+M_3 and APTS+M_4. Lane 4: dye **16**, **16**+M, **16**+M_2-3O, **16**+M_2-2O/**16**+M_2-4O (unresolved), **16**+M_3 and **16**+M_4. Lane 5: APTS, APTS-labelled 3'- and 6'-sialyllactoses. Lane 6: dye **16** and its conjugates with 3'- and 6'-sialyllactoses.

(lane 3, third spot from top). Conjugates **16**+M_2-2O and **16**+M_2-4O move as one spot, and conjugate **16**+M_2-3O moves faster (lane 4 in Figure 2). Each conjugate was also analyzed separately (Figure S6). Both dyes (APTS and **16**) easily resolve 3'- and 6'-isomers of sialyllactoses (lanes 5 and 6).

Outlook. The conjugates of the new dyes are ca. 3 times brighter than APTS derivatives (excitation with the 488 nm laser). The results obtained with dimers of mannose indicate that the selectivity profile of dye **16** is different from that of APTS, and this feature may be useful for the analysis of complex glycan mixtures. Figures 2, S5 and S6 show that all conjugates of dyes **6**-H and **16** are moving faster than the corresponding APTS analogs.[18] Therefore, dyes **6**-H and **16** with six negative charges may reveal "heavy" glycans undetectable with APTS due to very long retention times caused by the relatively low charge (-3) and the limited brightness. An access to the DNA sequencer with a CGE-LIF unit will enable to evaluate the crosstalk between the emission signals of APTS, on one hand, and the dyes **6**-H or **16**, on the other hand (also in conjugates), and their applicability for calibration of the retention times in CE.[4b]

Acknowledgements

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Keywords: fluorescent probes • glycoconjugates • electrophoresis • chromophores • donor-acceptor systems

[1] a) J. E. Turnbull, R. A. Field, *Nat. Chem. Biol.* **2007**, *3*, 74-77; b) R. D. Cummings, J. M. Pierce, *Chem. & Biol.* **2014**, *21*, 1-15; c) A. Varki, *Glycobiology*, **2017**, *27*, 3–49.

[2] a) *Essentials of Glycobiology*, Cold Spring Harbor Laboratory Press, **2009**, pp. 1-36, 101-114, 617-632, 661-678; b) S. Roseman, *J. Biol. Chem.* **2001**, *276*, 41527–41542.

[3] a) L. R. Ruhaak, G. Zauner, C. Huhn, C. Bruggink, A. M. Deelder, M. Wuhrer, *Anal. Bioanal. Chem.* **2010**, *397*, 3457–3481; b) K. Villadsen, M. C. Martos-Maldonado, K. J. Jensen, M. B. Thygesen, *ChemBioChem* **2017**, *18*, 574-612; c) L. R. Ruhaak, G. Xu, Q. Li, E. Goonatilleke, C. B. Lebrilla, *Chem. Rev.* **2018**, *118, 7886−7930*.

[4] a) M. S. Novotny, W. R. Alley, *Curr. Opin. Chem. Biol.* **2013** Oct., *17*(5), doi: 10.1016/j.cbpa.2013.05.029; b) G. Lu, C. L. Crihfield, S. Gattu, L. M. Veltri, L. A. Holland, *Chem. Rev.* **2018**, *118*, 7867–7885.

[5] a) A. Guttman, T. Pritchett, *Electrophoresis*, **1995**, *16*, 1906–1911; b) R. A. Evangelista, M.-S. Liu, F.-T. A. Chen, *Anal. Chem.* **1995**, 67, 2239 – 2245; c) W. Laroy, R. Contreras, N. Callewaert, *Nat. Prot.* **2006**, *1*, 397–405; d) N. Volpi, *Capillary electrophoresis of carbohydrates. From monosaccharides to complex polysaccharides*, Humana Press, N. Y. **2011**, pp. 1-51; e) N. Callewaert, S. Geysens, F. Molemans, R. Contreras, *Glycobiology* **2001**, *11*, 275– 281.

[6] a) H. Suzuki, O. Müller, A. Guttman, B. L. Karger, *Anal. Chem.* **1997**, *69*, 4554–4559; b) Z. Sharrett, S. Gamsey, L. Hirayama, B. Vilozny, J. T. Suri, R. A. Wessling, B. Singaram, *Org. Biomol. Chem.* **2009**, *7*, 1461–1470; c) L. R. Ruhaak, R. Hennig, C. Huhn, M. Borowiak, R. J. E. M. Dolhain, A. M. Deelder, E. Rapp, M. Wuhrer, *J. Proteome Res.* **2010**, *9*, 6655–6664; d) S.-C. Bunz, F. Cutillo, C. Neusüß, *Anal. Bioanal. Chem.* **2013**, *405*, 8277–8284.

[7] M. Pabst, D. Kolarich, G. Pöltl, T. Dalik, G. Lubec, A. Hofinger, F. Altmann, *Anal. Biochem.* **2009**, *384*, 263–273.

[8] a) C. Hansch, A. Leo, R. W. Taft, *Chem. Rev.* **1991**, *91*, 165–195; b) D. H. McDaniel, H. C. Brown, *J. Org. Chem.* **1958**, *23*, 420–427.

[9] H. Zollinger, W. Büchler, C. Wittwer, C. *Helv. Chim. Acta.* **1953**, *36*, 1711-1722; larger values for SO_3^- (σ_m = 0.30 and σ_p = 0.35) are mentioned in ref. 8a as a private communication of Viktor Palm.

[10] F. Bureš, *RSC Adv.* **2014**, *4*, 58826–58851.

[11] a) A. N. Butkevich, M. V. Sednev, H. Shojaei, V. N. Belov, S. W. Hell, *Org. Lett.* **2018**, *20*, 1261–1264; b) F. Wold, C. E. Ballou, *J. Biol. Chem.* **1957**, *227*, 301–312.

[12] Hydrolysis with aq. $Et_3N^*H_2CO_3$ buffer (after removal of $POCl_3$ and trimethyl phosphate) transforms *O*-alkyldichlorophoshates to primary alkyl phosphates and cleaves the N-P bond formed upon phorphorylation of the weakly nucleophilic arylamine.

[13] T. Li, R. Giasson, *J. Am. Chem. Soc.* **1994**, *116*, 9890–9893.

[14] a) T. Itoh, T. Mase, *Org. Lett.* **2004**, *6*, 4587–4590; b) C. Mispelaere-Canivet, J.-F. Spindler, S. Perrio, P. Beslin, *Tetrahedron*, **2005**, *61*, 5253-5259.

[15] H. S. Schultz, H. B. Freyermuth, S. R. Buc, *J. Org. Chem.* **1963**, *28*, 1140–1142.

[16] a) R. A. Evangelista, A. Guttman, F.-T. A. Chen, *Electrophoresis* **1996**, *17*, 347–351; b) L. R. Ruhaak, E. Steenvoorden, C. A. M. Koeleman, A. M. Deelder, M. Wuhrer, *Proteomics* **2010**, *10*, 2330-2336; c) F.-T. A. Chen, T. S. Dobashi, R. A. Evangelista, *Glycobiology* **1998**, *8*, 1045–1052.

[17] Yields were determined by integration of HPLC peak areas for the free dyes and their conjugates at isosbestic points; see Table S1 in Supporting Information.

[18] **6**-H+G moves slower than **APTS**+G (Figure S5).

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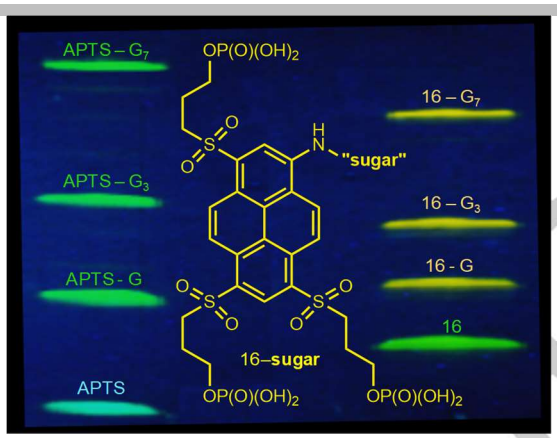
COMMUNICATION

Bright 1-aminopyrenes (ε ~ 20.000 $M^{-1}cm^{-1}$) with six negative charges (at pH ≥ 8) and high mobility in the electric field are designed for labeling and fluorescence detection of glycans related to the most structurally and functionally diverse class of natural products. Right and left lanes: new and reference dyes (lowest bands) and their conjugates.



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Negatively charged yellow-emitting 1-aminopyrene dyes for reductive amination and fluorescence detection of glycans