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The chemical reactivities of DOPA and dopamine derivatives and their regioselectivities upon oxidative nucleophilic trapping

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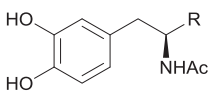
ABSTRACT

The chemical reactivities of four catecholamines, *N*-acetyl dopamine (NADA) and its dehydro derivative (NAΔDA), *N*-acetyl 3,4-dihydroxy-phenylalanine methyl ester (NADOPAME) and its dehydro derivative (NAΔDOPAME), under oxidative nucleophilic trapping and polymerisation conditions were compared and contrasted. Despite their structural similarities, varying reactivities and regioselectivities for oxidative nucleophilic trapping with ethanethiol were observed. This has possible implications on the use of these natural building blocks and their derivatives in the design and synthesis of biomimetic materials.

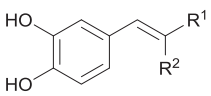
1. Introduction

Nature is remarkable in its ability to develop various materials for different functions and properties using seemingly simple building blocks. Such materials have been a source of inspiration for the design and synthesis of highly efficient and functional materials for various applications, e.g., functional coatings that are scratch resistant, materials for energy storage and so on. One of the most widely studied biomimetic materials is the material derived from dopamine (DA), a naturally occurring building block. The many applications of polydopamine are a source of continual wonder and have recently been reviewed.[1–7] In nature, *N*-acetyl dopamine (NADA) and *N*-acetyl dehydro dopamine (NAΔDA) are implicated in cuticular hardening.[8–10] Interestingly, a related catecholamine, dehydro 3,4-dihydroxy-phenylalanine is also a potential sclerotizing intermediate with its origin from L-3,4-dihydroxy-phenylalanine (L-DOPA).[11] Nature is 'economic' in this regard as these structurally related sclerotizing precursors can be derived from a common precursor tyrosine, and yet each gives rise to cuticles that have different hardness and colors.[8] This raises the interesting question as to how these catechols are different in terms of their reactivities and selectivities towards nucleophiles, which in turn may explain how these sclerotizing precursors can lead to materials of different hardness and strengths.

DOPA containing proteins have bioadhesive and sclerotizing functions in a number of invertebrates. In the former, DOPA proteins are known to be present in the attachment tendon of mussels,[2] and cements in certain Annelida species.[12,13] Their roles as sclerotizing precursors, e.g., in the egg case of parasitic trematodes, is responsible for the strength and durability of many structural materials found in nature.[12,14]



NADOPAME (1): R = COOMe
NADA (2): R = H



NAΔDOPAME (3): R^1 = COOMe, R^2 = NHAc
NAΔDA (4): R^1 = NHAc, R^2 = H

The oxidation chemistry of NADA[15–18] and NAΔDA[19–22] has been studied while that of NADOPAME[23] and NAΔDOPAME[23] is less explored. Despite these reports, the oxidation chemistry of these catecholamines cannot be easily compared in view of the varying conditions reported. Our interests stem from the desire

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to understand the chemistry of these catecholamines with the future goal of exploiting this in biomimetic material engineering. In this study, the reactivities of the four catecholamines towards oxidation, followed by nucleophilic trapping by ethanethiol are compared. The ease of polymerisation of these catecholamines under oxidative conditions was also investigated.

2. Results and discussion

2.1. Oxidative nucleophilic trapping studies of catecholamines

Our initial studies examined the oxidation of the catecholamines using three chemical oxidants, followed by in situ trapping with five equiv of ethanethiol. Oxidation of the catecholamines using sodium periodate was first attempted. Although this gave nearly full conversion to quinone (for NADOPAME and NADA as shown by NMR spectroscopy), the subsequent nucleophilic trapping by ethanethiol led to the formation of multiple products which could not be readily separated. In contrast, the use of silver oxide and DDQ as oxidants followed by trapping with ethanethiol gave cleaner reaction mixtures. Of the two oxidants, the use of silver oxide is more convenient as the oxidant can be filtered off prior to the addition of the nucleophile. This avoids complications associated with the presence of residual oxidants which may interfere in the oxidative nucleophilic trapping studies. The results are summarised in Table 1.

To rationalise the observed regioselectivities, attempts were made to identify the intermediates from oxidation. Thus, oxidation of each of the four catecholamines using silver oxide as oxidant was studied via spectrophotometric means (see ESD). For NADOPAME, NADA and NAΔDOPAME, the ortho-quinone was formed as evidenced by an absorption maximum of ca. 400 nm, as reported by others.[20,24,25] This ortho-quinone is relatively stable over 60 min. In the case of NAΔDA, the absorption maximum occurs at 485 nm which is the characteristic of a quinone methide[22] – this quinone methide imine amide is relatively unstable and decays rapidly over a 60-minute period. The formation of the quinone methide imine amide from the ortho-quinone of NAΔDA has been reported as a rapid enzymatic[22] as well as a chemical mediated isomerisation.[20] The analogous quinone methide imine amide of NAΔDOPAME was not observed. Schemes 1 and 2.

In the oxidative nucleophilic trapping studies with NADOPAME, NADA and NAΔDOPAME using silver oxide as the oxidant and ethanethiol as the nucleophile, a small amount of the starting catechol and the di-substituted adducts were isolated (Schemes 1 and 2). According to Huang et al., the oxidation potentials of NADA, its mono-substituted adduct, and its di-substituted adduct decrease in order of increasing substitution.[16] Hence, the observation above is believed to arise from the re-oxidation of the mono-substituted adduct by the intermediate catecholamine quinones, followed by a second nucleophilic substitution onto the quinone of the mono substituted adduct. To prove this, 1 equiv of 5-SEt NADA adduct (**6**) was added to freshly prepared NADA quinone in deuterated acetone. An immediate colour change was observed and the ^{1}H NMR spectrum of the reaction mixture after 3 min showed the presence of NADA (**2**) and 5-SEt NADA quinone. This is consistent with the notion of the redox reaction as shown in Scheme 3 and potentially explains the similar observations for the other three derivatives.

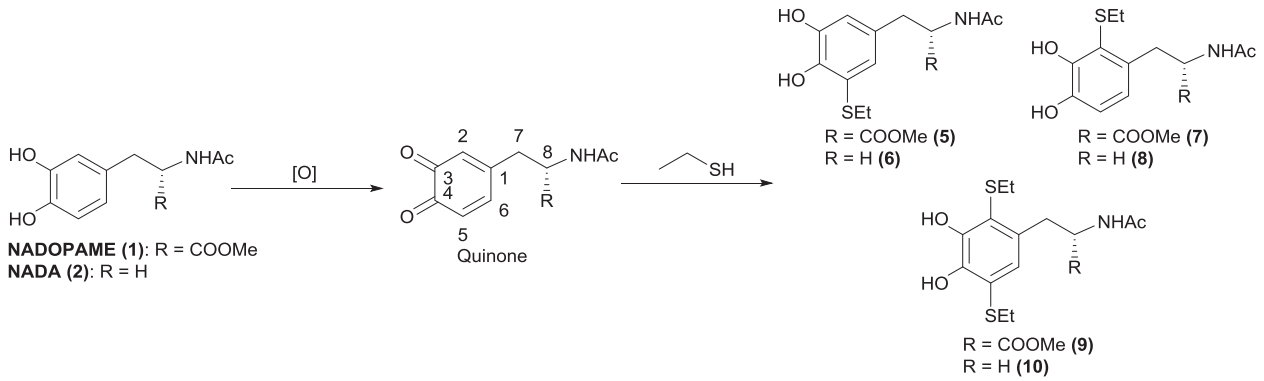
2.2. Semi-empirical calculations for predictions of regioselectivity

The semi-empirical method PM6[26] was used to calculate the LUMO coefficients of the atoms of both the ortho-quinone and the quinone methide or quinone methide imine amide for each of the catecholamines as summarised in Table 2 using Gaussian09.[27] In the case of NADOPAME quinone and NADA quinone, the size of the LUMO orbital coefficients are in the order of decreasing size, i.e., C-

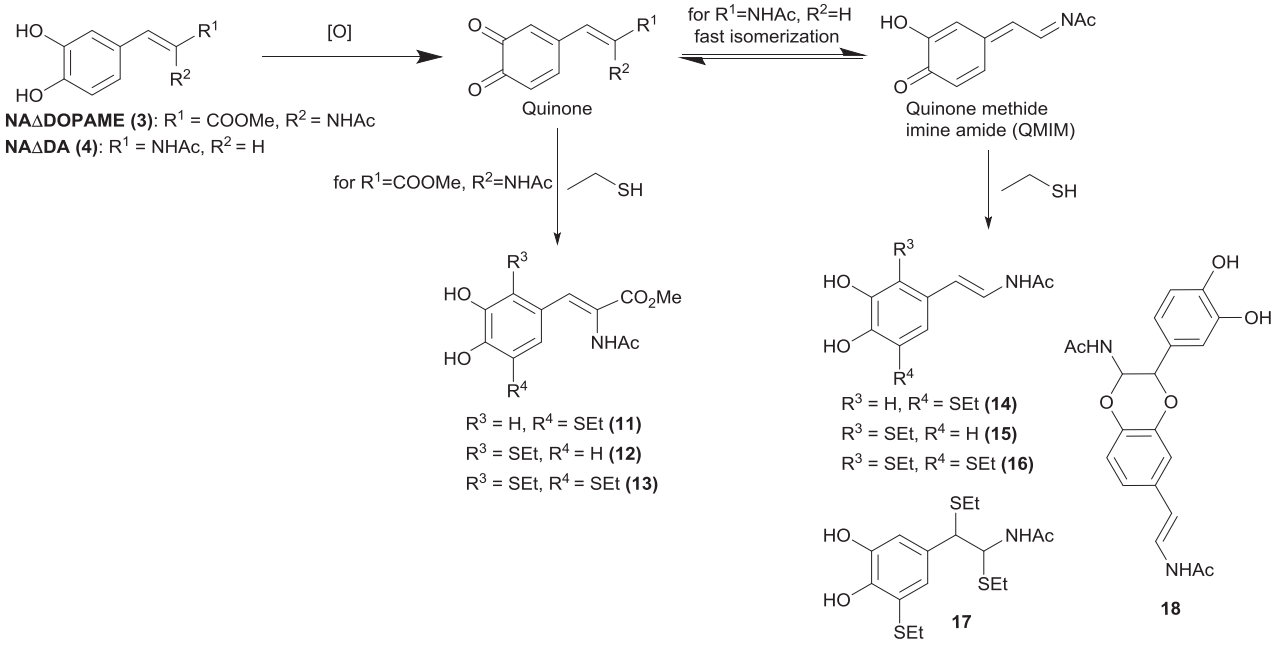
Table 1
Reaction conditions, ratio and yields for DOPA and DA derivatives under different oxidation conditions followed by addition of 5 equiv of ethanethiol

Catechol derivatives	Reaction conditions[a]	Ratio of compounds and isolated yield (recovered SM)
NADOPAME (**1**)	5 *eq.* Ag_2O, acetone, RT, 30 m	**5:7**=2:1, 80%, **9**=7% (**1**=10%)
	1.2 *eq.* DDQ, THF, −40 °C, 1 h	**5:7**=2:1, 81% (**1**=7%)
NADA (**2**)	5 *eq.* Ag_2O, acetone, RT, 30 m	**6:8**=7:1, 70%, **10**=3% (**2**=3%)
	1.2 *eq.* DDQ, THF, −40 °C, 1 h	**6:8**=6:1, 72%, **10**=1% (**2**=4%)
NAΔDOPAME (**3**)	5 *eq.* Ag_2O, acetone, RT, 30 m	**11:12**=1:5, 18%, **13**=2% (**3**=8%)
	1.2 *eq.* DDQ, THF, −40 °C, 1 h	**11:12**=3:7, 86% (**3**=13%)
NAΔDA (**4**)	5 *eq.* Ag_2O, acetone, RT, 10 m	**14:15:17:18**=1:2:2:3, 8% (**4**=35%)
	1.2 *eq.* DDQ, THF, −40 °C, 1 h	**16:17:18**=3:2:3, 8%
	1 *eq.* Ag_2O, MeOH, RT, 1 h[b]	**18**=40%

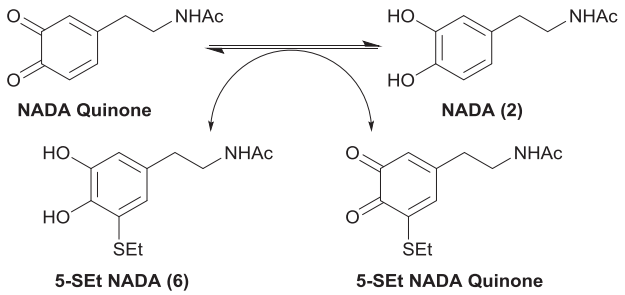
[a] Ethanethiol was added after the specified time.
[b] In the absence of ethanethiol, SM=starting material.



Scheme 1. Oxidative nucleophilic trapping studies with NADA and NADOPAME.



Scheme 2. Oxidative nucleophilic trapping studies with NAΔDA and NAΔDOPAME.



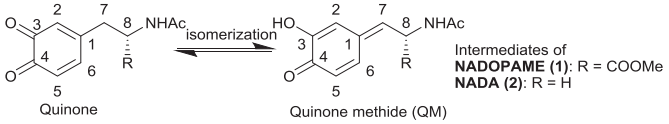
Scheme 3. Redox reaction between NADA quinone and its adduct.

1>C-6∼C-5>C-2 and C-1>C-6>C-5>C-2, respectively. The differences in the size of the orbital coefficients between C-2 and C-5 are slightly larger for NADA quinone than that for NADOPAME quinone which is consistent with the improved selectivity as tabulated in Table 1. However it should be noted that of the three available positions of attack, C-6 has the highest orbital coefficient for both the quinones of NADA and NAΔDOPAME but no C-6 addition adducts were observed in our studies. In contrast with NADOPAME and NADA, the C-2 of NAΔDOPAME quinone has the largest LUMO coefficient and this is consistent with the observation that the major adduct arises from addition of ethanethiol to the C-2 position. From the spectrophotometric studies above, the quinone methide imine amide of NAΔDA is the major intermediate from the oxidation reaction. Considering the orbital coefficients of this, the largest LUMO coefficient resides on the C-7 carbon. This is also consistent with experimental observations in which the formation of adducts **17** and **18** is only observed with NAΔDA.

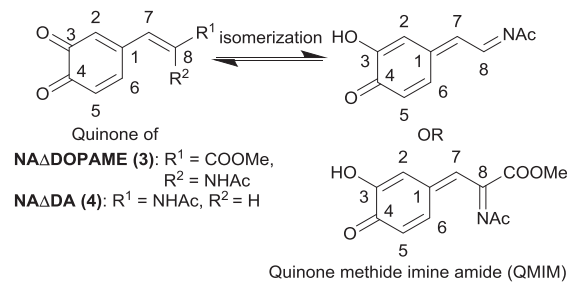
2.3. Oxidative polymerisation reactions of catecholamines

In order to examine the reactivity of each of the catecholamines under oxidative conditions, we examined the spectral changes of each compound upon oxidation with sodium periodate. Consistent with studies with silver oxide as the oxidant, the intermediates

Table 2
LUMO coefficients of quinones and quinone methides calculated using PM6



Atom	NADOPAME (**1**)		NADA (**2**)	
	Quinone	QM	Quinone	QM
C-1	0.38	−0.23	0.37	−0.27
C-2	−0.34	−0.21	−0.32	−0.24
C-3	−0.32	0.22	−0.32	0.30
C-4	−0.32	0.35	−0.33	0.36
C-5	−0.35	0.25	−0.35	0.30
C-6	0.35	−0.35	0.36	−0.38
C-7	−0.02	0.41	−0.01	0.47
C-8	0.06	0.06	0.05	−0.04



Atom	NAΔDOPAME (**3**)		NAΔDA (**4**)	
	Quinone	QMIM	Quinone	QMIM
C-1	−0.38	−0.35	0.38	−0.32
C-2	0.41	−0.19	−0.35	−0.20
C-3	0.30	0.28	−0.32	0.26
C-4	0.25	0.35	−0.31	0.35
C-5	0.29	0.27	−0.34	0.29
C-6	−0.26	−0.33	0.34	−0.34
C-7	−0.23	0.40	0.08	0.42
C-8	0.31	0.25	−0.28	0.25

ortho-quinone (λ_{max}~400 nm) and/or quinone methide (λ_{max}~485 nm) are observed for NADOPAME, NADA, NAΔDOPAME and NAΔDA, respectively (Fig. 1).

From these curves, the kinetics of the depletion of these intermediates can be delineated. The UV studies suggest that NAΔDOPAME quinone has a shorter half-life as compared to NADOPAME and NADA quinones. A separate study was carried out using in situ ^{1}H NMR monitoring at a concentration of 10 mM of the derivatives in D_2O, in the presence of 1 equiv of sodium periodate (for an example, see Fig. 2a). The NMR studies confirmed the identity of the ortho-quinones, and the disappearance of the quinone was measured relative to an internal standard i.e., DMSO.

Our studies show that the formation of all the ortho-quinones of NADA, NADOPAME and NAΔDOPAME occur very rapidly, i.e., within 3 min upon addition of the oxidant (the limit of ^{1}H NMR monitoring). For both NADA and NADOPAME, after an initial drop of the amounts of starting material to ca. 10%, with prolonged reaction time, the amounts of NADA and NADOPAME increased suggesting that a redox reaction has occurred between the cross-linked adduct and quinone that has regenerated the starting catecholamines (Fig. 2b). This phenomenon is depicted in Scheme 3. At 30 min, the amount of NADA was found to be 35% while NADOPAME was formed to a lesser extent, i.e., 19%. In the case of NAΔDOPAME(data not shown), only 50% of the quinone remained after ca. 3 min (with little or no starting catecholamine remaining) suggesting that the quinone had reacted more rapidly as compared to that of NADA and NADOPAME. This is also consistent with the observation of multiple signals in the ^{1}H NMR spectrum which signifies the formation of multiple adducts. In the case of NAΔDA oxidation monitored via UV-Vis spectroscopy, we observed a rapid isomerisation of the ortho-quinone from NAΔDA (λ_{max}=400 nm) to quinone methide (λ_{max}=485 nm) at neutral pH as was reported by Sugumaran et al.[19] The quinone methide imine amide was completely consumed within 2 min of formation. From these studies, the relative reactivities of the oxidation intermediates can be identified as NAΔDA quinone methide imine amide>NAΔDOPAME quinone>NADA quinone≈NADOPAME quinone.

The products of oxidation with sodium periodate were analysed by MALDI-TOF-TOF MS after 24 h. A dark brown suspension was observed for the polymerisation of NAΔDOPAME while a milky white suspension was obtained for NAΔDA. In contrast, no precipitate was observed in the polymerisation of NADOPAME and NADA and a clear reddish brown solution was observed instead. In cases where solids are formed, water was replaced with DMF to solubilize the solids formed, and filtered prior to analysis. Only the solids formed from DOPA and DA polymerisation did not fully dissolve in water and DMF or a mixture of both. The results are summarised in Table 3. In the analysis, the most prominent peak shown in the MALDI-TOF-TOF mass chromatogram was identified to be the most abundant 'oligomer' comprising 3 to 6 monomer units.

As the initial concentration of the monomers in the oxidative polymerisation is constant, the comparison of the amount of oligomer can be made based on the intensity of the signals shown in the MALDI-TOF-TOF MS analysis. The polymerisation of dopamine and DOPA afforded the most amount of oligomers. This is followed by NAΔDOPAME and NAΔDA while polymerisation of NADA and NADOPAME gave the lowest amount of oligomers. This is consistent with the observations that quinone intermediates that are more reactive lead to more oligomers.

In view of the interests in the use of polydopamine as coating materials, we also investigated the coating abilities of NADOPAME and NAΔDOPAME onto silicon wafer as substrate. AFM analysis of the coatings resulting from treatment of the catecholamines with 1 equiv of sodium periodate show that both NADOPAME and NAΔDOPAME are not able to coat on silicon wafer after oxidation. In

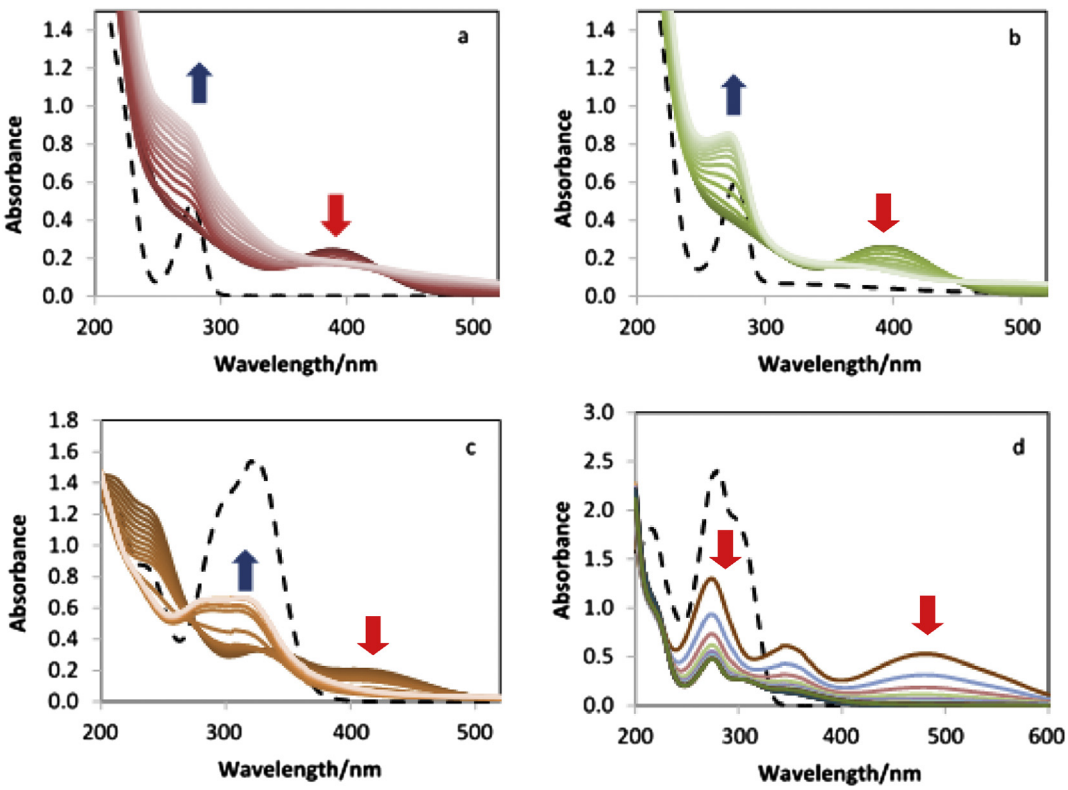


Fig. 1. Ultraviolet-visible spectral changes of (a) 0.2 mM NADOPAME (**1**), (b) 0.2 mM NADA (**2**), (c) 0.1 mM NAΔDOPAME (**3**), (d) 0.1 mM NAΔDA (**4**) associated with 1 equiv of $NaIO_4$-induced oxidation in water over 24 h. (Broken lines represent the starting materials' spectra and arrows indicate the spectral change over time).

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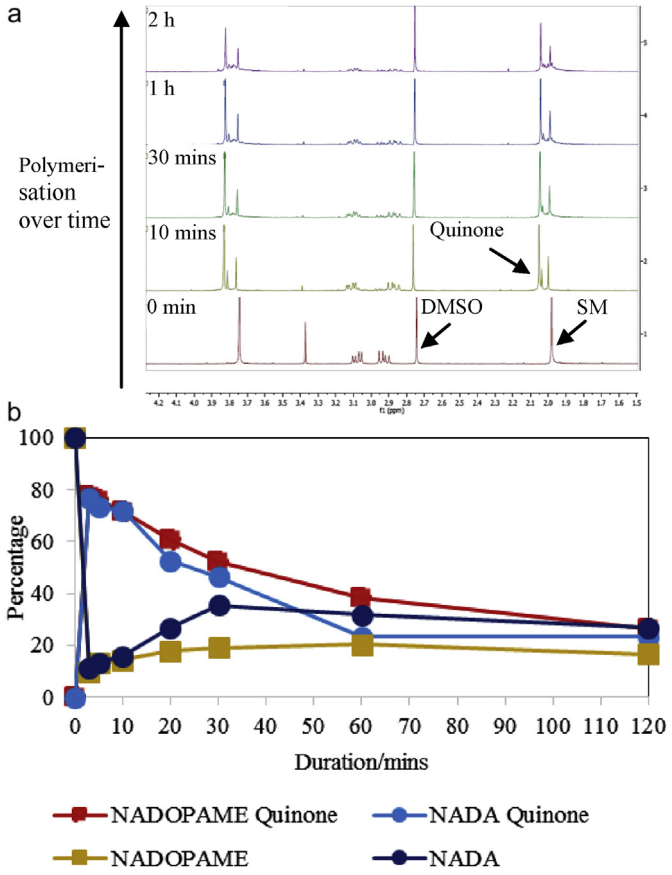

Fig. 2. (a)^{1}H NMR spectra monitoring of the oxidation of 10 mM NADOPAME with 1 equiv of $NaIO_4$ in D_2O (DMSO as the internal standard) for 2 h. (b) ^{1}H NMR monitoring of the amounts of NADA and NADOPAME (SM) and their respective quinones (Q) upon oxidation with $NaIO_4$ quantified by integration relative to an internal standard, DMSO.

Table 3
Molecular mass for the most prominent peak of each catecholamine derivatives with their signal intensity, using MALDI-TOF-TOF MS analysis, after oxidation with 1 equiv of $NaIO_4$ for 24 h

Monomers	Mass (monomer units[a])	Signal intensity (approx.)
Dopamine hydrochloride	877.0753 (~6)	66000
DOPA	1072.0661 (~5)	13000
NADOPAME (**1**)	810.2063 (~3)	900
NADA (**2**)	1020.3808 (~5)	1400
NAΔDOPAME (**3**)	1078.1127 (~4)	5200
NAΔDA (**4**)	1078.1210 (~6)	4400

[a] Monomer units=Mass/M_w of a monomer.

contrast, both DA and DOPA gave uneven coatings with 10–20 nm thickness under similar conditions (results not shown).

The four catecholamines are important precursors in oxidative polymerisation reactions, which have relevance in the context of structural materials, bioadhesives and even neurodegenerative diseases. It is especially of interest to note that seemingly similar catecholamines can give different reaction outcomes depending on the conditions and nucleophiles present. Our study on the chemical reactivities of these four catecholamines in oxidative nucleophilic trapping with thiols shows that the regiochemical outcomes of trapping can be significantly different. For example, the presence of a carboxymethyl group in NADOPAME as compared to that of NADA can affect the regioselectivity of thiol addition onto the quinone resulting in decreased regioselectivity. This is reflected in the slightly larger difference in the orbital coefficients between C-5 and C-2 in NADA quinone as compared to NADOPAME quinone.

However when considering PM6 calculations, the C-6 position for NADA quinone is larger than that of C-5 and C-2 while that for C-6 of NADOPAME quinone is comparable in size to C-5. Despite this, C6 addition adducts were not detectable in our studies, although the use of thiourea and nitrogen nucleophiles such as histidine in oxidative nucleophilic trapping experiments have been reported to give C-6 adducts as major products of reaction.[16–18] Huang et al. have attributed this to intramolecular base catalysis effects[16] but as discussed by Branco et al., this does not explain the differential regioselectivity at C2 and C5 positions.[18] What is evident is that the trapping by thiols such as ethanethiol is very rapid while trapping by nitrogen nucleophiles and thiourea are known to be much slower. The presence of unsaturation in both NADA and NADOPAME gives rise to more reactive oxidation intermediates than the corresponding saturated systems. Intriguingly, the quinone of NAΔDA rapidly isomerises to the quinone methide imine amide which is highly reactive and nucleophilic trapping occurs predominantly at the β-positions i.e., the side chain. In contrast, the quinone methide of NAΔDOPAME is not observed in our experiments, and thiol trapping of the quinone of NAΔDOPAME favours C-2 ring addition over C-5 ring addition. The latter is consistent with predictions based on orbital coefficients from PM6 calculations.

The oxidative polymerisation studies of the four catecholamines were compared to that of dopamine and DOPA. In all the cases, oligomers ranging from 3 to 6 repeating units are detected in the MALDI-TOF-TOF-MS with DA and DOPA giving the highest amount of oligomers. This suggests that the presence of a free amine in the catecholamines is critical for efficient oxidative polymerisation reactions. This is also evident from the unsuccessful attempts to coat silicon wafers with NADOPAME and NAΔDOPAME as compared to DA and DOPA under sodium periodate oxidative conditions.

3. Conclusions

The comparative chemical reactivity studies with the four catecholamines under oxidative nucleophilic/polymerisation conditions demonstrate the economy of nature's building blocks. Although the catecholamines are structurally very similar, the reactivities and regioselectivities can vary under similar reaction conditions, leading to different adducts/polymers. It is anticipated that the positional isomers of adducts or polymers will have different properties due to the crosslinking and overall stacking of the repeating units, affecting bulk properties of these materials. In this context, one should be able to exploit these chemistries in designing biomimetic functional materials as well as tune the properties of the materials through judicious choice of catecholamines, conditions and nucleophiles.

4. Experimental

4.1. Materials and methods

NADA,[28] NAΔDA,[29–31] NADOPAME[32,33] and NAΔDOPAME[34] were synthesised following reported literature procedures. All other reagents and solvents were purchased from Sigma Aldrich or Alfa Aesar and were used without further purification unless otherwise specified. Silver(I) oxide was freshly prepared by adding 1 equiv of silver nitrate solution to a solution of sodium hydroxide. Reactions involving air or moisture sensitive reagents were performed with dried glassware under a nitrogen atmosphere. Thin layer chromatography (TLC) was performed on Merck precoated silica gel plates. Visualization was accomplished with UV light. Compounds were purified by flash chromatography on a column

using Merck silica gel 60 (230–400 mesh) or semi-preparative reversed-phase HPLC/PDA method using Gemini® 5 μm C18 110 Å, LC Column 150×10 mm. Mass spectra were recorded on an Applied Biosystems MDS SCIEX API 2000 mass spectrometer. High resolution mass spectra (HRMS) were recorded on an Agilent Mass spectrometer or Bruker micrOTOFQII using ESI-TOF (electrospray ionization-time of flight). NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer at 400 MHz for ^{1}H and at 100 MHz for ^{13}C on a Bruker spectrometer with methanol-d_4 or acetone-d_6 as solvent. The chemical shifts are given in ppm, using the proton solvent residue signal (CD_3OD: δ 3.31, acetone-d_6: δ 2.05) as a reference in the ^{1}H NMR spectrum. The deuterium coupled signal of the solvent was used as a reference in ^{13}C NMR (CD_3OD: δ 49.00). The following abbreviations were used to describe the signals: s=singlet, d=doublet, t=triplet, m=multiplet, q=quartet. IR spectra were recorded on a Perkin–Elmer Spectrum 100 FTIR spectrometer.

4.2. General procedure for oxidation nucleophilic trapping study

5 equiv of Ag_2O were added to a stirred solution of catecholamines in acetone at room temperature. After 30 min (10 min for NAΔDA), Ag_2O was removed from the yellow reaction mixture via filtration. To the filtrate, 5 equiv of ethanethiol were added. Stirring was continued for 1 h and then the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (5% MeOH/DCM) and/or semi-preparative reversed-phase HPLC/PDA method. All the purified compounds are brown oil unless specified otherwise.

1.2 equiv of DDQ were added to a solution of catecholamines in dry THF at −40 °C under nitrogen. The reaction mixture was stirred at −40 °C for 1 h and then 5 equiv of ethanethiol were added. Stirring was continued at same temperature for 1 h and at room temperature for 2.5 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (5% MeOH/DCM) and/or semi-preparative reversed-phase HPLC/PDA method.

4.3. Characterisation of adducts

*4.3.1. Methyl (S)-2-acetamido-3-(3-(ethylthio)-4,5-dihydroxy-phenyl)propanoate (**5**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.39; ν_{max} (liquid film) 3349 (br), 2960, 1735, 1649, 1430, 1364, 1220 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.66 (d, J=2.0 Hz, 1H), 6.58 (d, J=2.0 Hz, 1H), 4.58 (dd, J=8.4, 5.9 Hz, 1H), 3.69 (s, 3H), 2.97 (dd, J=13.9, 5.9 Hz, 1H), 2.88–2.72 (m, 3H), 1.92 (s, 3H), 1.21 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.6, 173.1, 146.3, 145.2, 129.4, 125.4, 122.1, 116.6, 55.5, 52.7, 37.8, 28.9, 22.3, 15.0. HRMS (ESI): m/z calcd for $C_{14}H_{19}NNaO_5S$ $[M+Na]^+$ 336.0876, found 336.0878.

*4.3.2. Methyl (S)-2-acetamido-3-(2-(ethylthio)-3,4-dihydroxy-phenyl)propanoate (**7**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.43; ν_{max} (liquid film) 3355 (br), 2957, 1732, 1647, 1428, 1372, 1219 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.71 (d, J=8.0 Hz, 1H), 6.58 (d, J=8.0 Hz, 1H), 4.67 (dd, J=8.6, 6.6 Hz, 1H), 3.64 (s, 3H), 3.33–3.36 (m, 1H), 3.05 (dd, J=13.5, 8.6 Hz, 1H), 2.78 (m, 2H), 1.90 (s, 3H), 1.17 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.9, 173.1, 148.2, 145.3, 132.9, 122.3, 120.8, 116.5, 55.1, 52.5, 37.2, 30.1, 22.3, 14.9. HRMS (ESI): m/z calcd for $C_{14}H_{19}NNaO_5S$ $[M+Na]^+$ 336.0876, found 336.0880.

*4.3.3. Methyl (S)-2-acetamido-3-(2,5-bis(ethylthio)-3,4-dihy-droxyphenyl)propanoate (**9**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.50; ν_{max} (liquid film) 3351 (br), 2960, 2938, 1737, 1657, 1436, 1371, 1227 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.74 (s, 1H), 4.70 (dd, J=8.8, 6.3 Hz, 1H), 3.66 (s, 3H), 3.37 (dd, J=13.5, 6.3 Hz, 1H), 3.03 (dd, J=13.5, 8.8 Hz, 1H), 2.85 (q, 2H), 2.77 (m, 2H), 1.90 (s, 3H), 1.24 (t, 3H), 1.18 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.8, 173.0, 147.9, 144.5, 133.0, 124.9, 123.6, 119.8, 54.9, 52.6, 37.1, 30.3, 28.3, 22.3, 15.0, 14.9. HRMS (ESI): m/z calcd for $C_{16}H_{23}NNaO_5S_2$ $[M+Na]^+$ 396.0910, found 396.0921.

*4.3.4. N-(3-(Ethylthio)-4,5-dihydroxyphenethyl)acetamide (**6**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.31; ν_{max} (liquid film) 3299 (br), 2928, 1631, 1428, 1372 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.69 (d, J=2.0 Hz, 1H), 6.60 (d, J=2.0 Hz, 1H), 3.33 (t, 2H), 2.82 (q, 2H), 2.62 (t, 2H), 1.90 (s, 3H), 1.22 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.2, 146.3, 144.7, 132.0, 125.0, 122.0, 116.3, 42.2, 35.8, 29.0, 22.5, 15.0. HRMS (ESI): m/z calcd for $C_{12}H_{17}NNaO_3S$ $[M+Na]^+$ 278.0821, found 278.0823.

*4.3.5. N-(2-(Ethylthio)-3,4-dihydroxyphenethyl)acetamide (**8**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.34; ν_{max}(liquid film) 3299 (br), 2927, 1627, 1554, 1480, 1426 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.74 (d, J=8.2 Hz, 1H), 6.65 (d, J=8.2 Hz, 1H), 3.37–3.30 (m, 2H), 2.99–2.93 (m, 2H), 2.76 (q, 2H), 1.90 (s, 3H), 1.17 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.3, 148.0, 144.7, 135.5, 121.6, 120.4, 116.8, 42.1, 34.8, 30.1, 22.6, 14.9. HRMS (ESI): m/z calcd for $C_{12}H_{17}NNaO_3S$ $[M+Na]^+$ 278.0821, found 278.0814.

*4.3.6. N-(2,5-Bis(ethylthio)-3,4-dihydroxyphenethyl)aceta-mide (**10**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.42; ν_{max} (liquid film) 3323 (br), 2961, 2927, 1643, 1448, 1374, 1269 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.78 (s, 1H), 3.37–3.32 (m, 2H), 2.99–2.94 (m, 2H), 2.87 (q, 2H), 2.75 (q, 2H), 1.91 (s, 3H), 1.25 (t, 3H), 1.17 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.2, 147.8, 143.9, 135.5, 124.2, 124.0, 119.3, 42.0, 34.8, 30.3, 28.2, 22.6, 14.9 (2C). HRMS (ESI): m/z calcd for $C_{14}H_{21}NNaO_3S_2$$[M+Na]^+$ 338.0855, found 338.0854.

*4.3.7. Methyl (Z)-2-acetamido-3-(3-(ethylthio)-4,5-dihydroxy-phenyl)acrylate (**11**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.31; ν_{max} (liquid film) 3318 (br), 2955, 2927, 1705, 1664, 1484, 1436 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.31 (s, 1H), 7.13 (d, J=1.9 Hz, 1H), 7.06 (d, J=1.9 Hz, 1H), 3.79 (s, 3H), 2.86 (q, 2H), 2.13 (s, 3H), 1.24 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.3, 167.4, 148.3, 146.2, 136.3, 127.2, 126.3, 124.0, 123.0, 116.7, 52.8, 28.5, 22.6, 14.9. HRMS (ESI): m/z calcd for $C_{14}H_{17}NNaO_5S$ $[M+Na]^+$ 334.0720, found 334.0717.

*4.3.8. Methyl (Z)-2-acetamido-3-(2-(ethylthio)-3,4-dihydroxy-phenyl)acrylate (**12**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.34; ν_{max} (liquid film) 3369 (br), 1701, 1636, 1437, 1364, 1231 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.97 (s, 1H), 7.11 (d, J=8.4 Hz, 1H), 6.82 (d, J=8.4 Hz, 1H), 3.81 (s, 3H), 2.76 (q, 2H), 2.04 (s, 3H), 1.16 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.2, 167.6, 148.2, 147.6, 134.8, 129.9, 125.5, 122.3, 122.2, 116.6, 52.9, 30.5, 22.4, 15.1. HRMS (ESI): m/z calcd for $C_{14}H_{18}NO_5S$ $[M+H]^+$ 312.0901, found 312.0896.

*4.3.9. Methyl (Z)-2-acetamido-3-(2,5-bis(ethylthio)-3,4-dihy-droxyphenyl)acrylate (**13**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.41; ν_{max} (liquid film) 3324 (br), 2957, 2924, 1708, 1665, 1436, 1228 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.96 (s, 1H), 7.22 (s, 1H), 3.82 (s, 3H), 2.86 (q, 2H), 2.74 (q, 2H), 2.05 (s, 3H), 1.27 (t, 3H), 1.16 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.1, 167.4, 147.8, 146.4, 134.6, 129.9, 125.8, 124.5, 123.7, 120.7, 52.9, 30.8, 27.9, 22.5, 15.1, 15.0. HRMS (ESI): m/z calcd for $C_{16}H_{21}NNaO_5S_2$$[M+Na]^+$ 394.0753, found 394.0756.

*4.3.10. (E)-N-(3-(Ethylthio)-4,5-dihydroxystyryl)acetamide (**14**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.27; ν_{max} (liquid film) 3340 (br), 2962, 1636, 1260 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.24 (d, J=14.7 Hz, 1H), 6.77 (d, J=1.8 Hz, 1H), 6.75 (d, J=1.8 Hz, 1H), 6.03 (d, J=14.7 Hz, 1H), 2.83 (q, 2H), 2.03 (s, 3H), 1.23 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 170.7, 146.5, 145.5, 129.8, 122.7, 122.4, 122.2,

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114.3, 112.3, 28.9, 22.5, 14.9. HRMS (ESI): *m/z* calcd for $C_{12}H_{15}NNaO_3S$ $[M+Na]^+$ 276.0665, found 276.0659.

*4.3.11. (E)-N-(2-(Ethylthio)-3,4-dihydroxystyryl)acetamide (**15**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.30; ν_{max} (liquid film) 3360 (br), 2916, 2850, 1656, 1633, 1470, 1418 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.26 (d, J=14.7 Hz, 1H), 6.95 (d, J=8.4 Hz, 1H), 6.87 (d, J=14.7 Hz, 1H), 6.77 (d, J=8.4 Hz, 1H), 2.69 (q, 2H), 2.04 (s, 3H), 1.14 (t, 3H). HRMS (ESI): *m/z* calcd for $C_{12}H_{15}NNaO_3S$ $[M+Na]^+$ 276.0665, found 276.0668.

*4.3.12. (E)-N-(2,5-Bis(ethylthio)-3,4-dihydroxystyryl)aceta-mide (**16**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.40; ν_{max} (liquid film) 3340 (br), 2962, 2922, 1635, 1260 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.28 (d, J=14.7 Hz, 1H), 7.04 (s, 1H), 6.84 (d, J=14.7 Hz, 1H), 2.91 (q, J=7.4 Hz, 2H), 2.68 (q, 2H), 2.05 (s, 3H), 1.27 (t, 3H), 1.14 (t, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 170.7, 147.6, 143.9, 133.4, 125.0, 123.6, 119.0, 117.7, 113.1, 30.4, 28.0, 22.6, 14.9, 14.8. HRMS (ESI): *m/z* calcd for $C_{14}H_{19}NNaO_3S_2$ $[M+Na]^+$ 336.0699, found 336.0694.

*4.3.13. N-(1,2-Bis(ethylthio)-2-(3-(ethylthio)-4,5-dihydroxy-phenyl) ethyl)acetamide (**17**).* Oil; R_f (10% CH_3OH/CH_2Cl_2) 0.50; ν_{max} (liquid film) 3260 (br), 2969, 2928, 1655, 1583, 1421 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.83 (d, J=2.1 Hz, 1H), 6.80 (d, J=2.1 Hz, 1H), 5.34 (d, J=8.8 Hz, 1H), 3.97 (d, J=8.8 Hz, 1H), 2.83 (q, 2H), 2.54–2.26 (m, 4H), 1.99 (s, 3H), 1.27–1.08 (m, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 172.7, 146.2, 145.8, 131.9, 125.4, 121.7, 116.0, 59.2, 55.2, 28.8, 26.3, 25.9, 22.5, 15.2, 15.0, 14.8. HRMS (ESI): *m/z* calcd for $C_{16}H_{25}NNaO_3S_3$ $[M+Na]^+$ 398.0889, found 398.0887.

*4.3.14. (E)-N-(2-(2-Acetamido-3-(3,4-dihydroxyphe-nyl)-2,3-dihy-drobenzo[b][1,4]dioxin-6-yl)vinyl)acetamide (**18**).* 1 equiv of Ag_2O was added to a stirred solution of NAΔDA in methanol at room temperature. After 1 h, Ag_2O was removed from the yellow reaction mixture via filtration. The residue was purified by silica gel column chromatography (5% MeOH/DCM) to yield 40% of **18** as a solid. Mp 182–184 °C; R_f (10% CH_3OH/CH_2Cl_2) 0.23; ν_{max} (liquid film) 3265, 2924, 1642, 1498, 1371, 1260 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.30 (d, J=14.7 Hz, 1H), 6.92–6.70 (m, 6H), 6.10 (d, J=14.7 Hz, 1H), 5.69 (d, J=7.2 Hz, 1H), 4.71 (d, J=7.2 Hz, 1H), 2.03 (s, 3H), 1.87 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 173.2, 170.6, 147.2, 146.5, 144.6, 142.6, 131.9, 128.7, 122.1120.6, 120.3, 118.2, 116.1, 115.6, 114.7, 114.0, 78.4, 78.4, 22.6, 22.6. HRMS (ESI): *m/z* calcd for $C_{20}H_{20}N_2NaO_6$ $[M+Na]^+$ 407.1214, found 407.1218.

4.4. Procedure for UV-Vis monitoring of oxidation of catecholamines with 5 equiv of silver oxide in acetone

Catecholamines were dissolved in 1 ml of acetone to make up 10 mM followed by stirring with 5 equiv of silver (I) oxide. 28 μL of the reaction mixtures were withdrawn and diluted to 0.2 mM at different time points and subjected to UV/Vis analysis using a Shimadzu UV-1800 UV-Visible Spectrophotometer.

4.5. Procedure for monitoring the polymerisation of catecholamines with sodium periodate

4.5.1. UV-Vis absorption spectroscopy. 0.2 mM of catecholamines (NADOPAME and NADA) or 0.1 mM of catecholamines (NAΔDOPAME and NAΔDA) were oxidised by 1 equiv of $NaIO_4$ in water. The reaction mixtures in quartz cuvettes were analysed by a Shimadzu UV-1800 UV-Visible Spectrophotometer at various time points for 24 h.

4.5.2. ^1H NMR spectroscopy. These experiments were performed with 10 mM D_2O solutions of catecholamine derivatives (spiked with 0.2 mM of DMSO as an internal standard) at room temperature in NMR tubes. An equal volume of 1 equiv of $NaIO_4$ in D_2O was added to a solution of 20 mM of catecholamine derivatives. Subsequently, the samples were analysed by ^1H NMR spectroscopy at various time points (3, 5, 10, 20, 30, 60, 120 min).

4.5.3. MALDI-TOF-TOF-MS. Oxidation of catecholamine derivatives was carried out by mixing 0.5 ml of 20 mM monomers in water with 0.5 ml of 20 mM $NaIO_4$ in water. The reaction mixtures were stirred for 24 h at room temperature and filtered. Those mixtures with suspended solids were reconstituted with 1 ml of DMF prior to the filtration. 0.5 μL of the filtrates was spotted on a 384 well target plate and crystallized with 0.5 μL of α-cyano-4-hydroxycinnamic acid (CHCA) in 0.1% (v/v) Trifluoroacetic Acid (TFA) and 50% (v/v) Acetonitrile (ACN), which were subsequently subjected to MALDI-TOF/TOF mass analysis (4800 MALDI TOF/TOF Analyzer from Applied Biosystems, Framingham, MA, USA). The *m/z* data was manually acquired in the reflector mode by using the Reflectron Method (Accelerating Voltage: 20000V, Laser Intensity: 3300–3600).

4.6. Coating studies

The substrate silicon wafer (size: 10 mm×10 mm), was immersed in 1.5 ml of reaction mixture consisting of 10 mM monomers and in water for 24 h at room temperature. Subsequently, they were subjected to AFM analysis. The characterization was carried out on Bruker Dimension ICON.

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Supplementary data

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